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Effects of an auxin, cytokinins, and genotype on in vitro propagation of Impatiens sp. 'T63-1' and 'Starfire'

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I. INTRODUCTION

The group of exotic ornamentals known as the New Guinea Impatiens is new to the U.S.A. and has created much interest among gardeners and plant breeders (Arisumi and Cathey 1976, and Winters 1973). Certain Impatiens species from New Guinea comprise a group of plants having a wealth of ornamentally desirable foliage and floral traits (Martin 1984 and Arisumi and Cathey 1976). The flowers range from 2.5 cm across, with colors from white to various pastel shades of pink, lavender, and orange to magenta, scarlet, and vermillion. The leaves are of various sizes and shapes from dull to bright green above and from pale to dark red below, with and without variegation. Leaf and stem color varies from green to intense dark red (Arisumi and Cathey 1976, Arisumi 1973, and Winters 1973).

Several recent Impatiens plant introductions (P.I.s) from New Guinea possess a wide array of leaf variegation and larger flowers than the non-variegated species (USDA 1974 and Winters 1973). Java Impatiens, I. platypetala, generally have more flowers and grow better under adverse environmental conditions than New Guinea Impatiens. New Guinea Impatiens comprise probably 4 interbreeding subspecies from that island (Pasutti 1977), so these will be collectively referred to as Impatiens sp. The Celebes species, I. platypelata subsp. aurantiaca, is probably a separate species from those of New Guinea and Java (Pasutti 1977). Java x New Guinea and Celebes x New Guinea interspecific hybrids often exhibit the desirable charateristics of both species (Stephens et al. 1985, Arisumi 1974, and Pasutti and Weigle 1980).

The P.I.s were collected in the subtropical highlands of Australian New Guinea in 1970 by H. F. Winter and J. J. Higgins of the Agricultural Research Service who led an expedition for ornamental plant exploration in New Guinea (Martin 1984, Arisumi and Cathey 1976, Woodroffe 1975, and Winters 1973). In 1972, the first hybrid New Guinea Impatiens were developed by Pennsylvania's Longwood Gardens, and the Plant Introduction Station, USDA, distributed the plants to various research institutions, commercial growers, and amateur breeders throughout the country (Martin 1984, Armstrong 1974, and Winters 1973). Besides the Longwood Gardens, Iowa State University and the Ornamental Laboratory, USDA, have also produced many hybrids as well. Iowa State University, for example, issued a group known as the Star series, which have variegated leaves and deep-violet flowers (Martin 1984, Arisumi and Cathey 1976, and Woodroffe 1975).

New Guinea Impatiens are gaining increased acceptance as ornamental spring pot and bedding plants, available from retail greenhouses via specialized propagators (Stephens et al. 1985, Arisumi and Cathey 1976, and Winters 1973). According to a recent survey, Impatiens are now this country's most popular bedding plant, a title they stole in 1978 from petunias, which have been losing ground ever since (Martin 1984 and Winters 1973). During the past decade, Impatiens have become increasingly important in commerce. The National Bedding Plant Producers Association estimates that Impatiens plants account for 2-3 % of all bedding plants, or more than $3,000,000 annually (Martin 1984 and Winters 1973).

Like many other species of the genus, the New Guinea Impatiens and interspecific hybrids can be vegetatively propagated by cuttings (Arisumi

and Cathey 1976, Stephens *et al.* 1985, Armstrong 1974, and Winters 1973). But, cutting production often becomes limiting on greenhouse-grown plants since flower bud initiation and development occur throughout the year. Reproductive stem tips root more slowly than vegetative stem tips (Stephens *et al.* 1985). To produce a large number of a clone in a short time, and to preserve the full genetic characteristics of each clone, which appears to have superior form and rapid growth rate, we might be able to apply tissue culture techniques. Theses techniques have, in recent years, developed into very powerful tools for propagation of ornamental species. Hughes (1982) suggested that the *in vitro* multiplication of ornamental plants had the following advantages over conventional methods of plant propagation: 1) the number of genetically identical plants from a single stock plant is greatly increased, 2) disease free plants may be obtained, 3) stocks may be maintained *in vitro*, 4) the techniques may be used to obtain plants from crosses between incompatible species through either embryo or ovule culture, and 5) in a few ornamental species, haploid plants have been obtained through anther culture. Although several excellent reviews as well as many papers concerning the application of tissue culture technology to orchid and other ornamental species are available, only a few papers concerning the application of this technology to *Impatiens* sp. have been published (Koenigsberg and Langhans 1976, Arisumi 1985, and Stephens *et al.* 1985). More research is needed on *Impatiens* sp. for the establishment of an efficient tissue culture technology.

This thesis describes the effects of growth regulators (NAA and 3

cytokinins) on micropropagation of 2 Impatiens hybrids during culture in vitro. Experiment 1 deals with 'T63-1', which is a hybrid of a cross between Java x New Guinea Impatiens. Experiment 2 deals with 'Starfire', a hybrid of a Celebes x New Guinea Impatiens cross. Finally, Experiment 3 deals with both 'T63-1' and 'Starfire', in order to determine the effect of genotype.

II. LITERATURE REVIEW

A. Brief Historical Development of Plant Tissue Culture

1. Introduction

Plant tissue culture techniques have become powerful tools for studying basic and applied problems in plant physiology, genetics, plant biology, biochemistry, anatomy, plant pathology, etc. Furthermore, in the last twenty years these techniques have found wide commercial application in the propagation of plants, particularly horticultural species. These techniques are expected to become more developed for commercial applications as well as contributing to basic and applied research in many fields.

2. Early studies in plant tissue culture

Even though it is not known when the first attempts were made to vegetatively propagate plants, plant propagation through tissue culture techniques may have been conducted as early as 1902 when Haberlandt first attempted to regenerate plants from single cells (Conger 1982 and Murashige 1974). Haberlandt in 1902 stated clearly the desirability of culturing the isolated vegetative cells of higher plants. Although he failed to regenerate plants from single cells, he inspired others to attempt *in vitro* culture of plant tissues (Street 1977 and Conger 1982). Very little progress was made during the 30 years following Haberlandt's paper even though Kotte (1922a,b) and Robins (1922a,b) reported some progress towards the culture of excised root tips.

White (1934) reported the successful establishment of an actively growing clone from tomato root tips which were excised and grown in a simple liquid medium of known constitution. In the same year Gautheret (1934) also reported that pieces of cambium removed under aseptic conditions from Salix capraea and some other trees continued to proliferate in a solidified medium containing Knop's solution, glucose, and cysteine hydrochloride. Gautheret (1939) and White (1939) independently reported similar results in the same year. Gautheret studied small explants from carrot roots using a culture medium containing glucose, vitamin B1, cysteine hydrochloride and IAA. White studied the formation of a similar tissue culture from the procambial tissue of hybrid Nicotiana glauca x N. langsdorffii using a culture medium which was the same as that he had employed for the actively growing clone from tomato root tips (White 1934). They reported that their cultures were successfully subcultured. Their papers became the first report for the prolonged culture of unorganized plant tissue such as callus. The basic techniques described in their papers have been subsequently applied to many other species.

3. Discovery of growth regulators

The experiment of Miller and Skoog (1953) on bud formation from cultured pith explants of tobacco led them into the discovery of kinetin (Miller et al. 1956). Skoog and Miller (1957) also reported that the auxin/cytokinin ratio within the concentrations tested affected morphological changes in growth of tobacco. However, no universial ratio

of auxin and cytokinin exists for morphological changes (Hughes 1982). Quantitative interaction between auxin and cytokinin provided a common mechanism for the regulation of all types of growth. In 1958 Steward et al. reported organ development in the cultured mass of carrot cells from the growth of freely suspended cells. He demonstrated that normal roots arose in the liquid medium, and the cultures developed shoots when they were placed on a semisolid agar medium.

4. Haploid induction through tissue culture techniques

Melchers and Bergmann (1959) cultivated tissue derived from a haploid shoot of Antirrhinum majus. The tissue retained its haploid state during several subcultures but then increased in ploidy. Haploid tissue and suspension cultures are clearly of particular interest for those interested in studying mutation and plant breeding. Nitsch and Nitsch (1969) reported a method to grow hundreds of tobacco haploid plants from pollen grains. When they were grown in vitro on a relatively simple medium, some pollen grains proliferated into embryo-like structures which developed in stages similar to those of zygotic embryos but seed was not set. The induction of halploid plants from anther and pollen culture is of great interest in plant breeding since recessive mutations induced in them can be identified immediately, and doubling of the chromosomes leads directly to homozygous individuals. For example, induction and isolation of mutants from sugarcane tissue culture which are resistant to various diseases have been especially successful (Heinz et al. 1977 and Liu 1981,1984).

5. Protoplast culture

Cocking (1960) first reported the release of plant protoplasts using an extract of hydrolytic enzymes to isolate protoplasts from tobacco root tips. Since that time many enzyme formulations have been used to isolate protolasts. Takebe et al. (1971) showed, for the first time, that protoplasts isolated from mesophyll cells of tobacco could be induced to generate entire plants, which differentiated normally but frequently had abnormal chromosome numbers obviously connected with sterility and abnormality in form. Power et al. (1970) reported that protoplasts could be stimulated to fuse under defined experimental conditions. One of the most important events was that of Carlson et al. (1972), who reported the successful production of the first interspecific somatic hybrid plants from two tobacco species by protoplast fusion. The biochemical and morphological characteristics of the somatically produced amphiploid were identical to those of the sexually produced amphiploid. Although there have now been many reports of successful interspecific and intergenetic hybrids (Dudits et al. 1979 and Krumbiegel and Schieder 1979) the number of species in which plants can be regenerated from protoplasts are limited. Unfortunately, a large gap between expectation and performance has plagued protoplast research during the 1970s and early 1980s. Nevertheless protoplast culture is still of great interest for modifying the plant's genome by uptake of exogenous DNA and creating hybrids between sexually incompatible species through protoplast fusion (Hughes 1982).

6. Somatic embryogenesis

Steward (1958) and Reinert (1958 and 1959) first reported initiation and development of embryos from somatic tissues of carrot. Since 1958, there have been many studies on somatic embryogenesis in both cultivated and wild varieties (Esan 1973 and Lakshmi Sita et al. 1980). Murashige (1978) reported that asexual or somatic embryogenesis was potentially the most rapid method of cloning plants in vitro but this method as presently known was not a desired way to propagate plants. However, Ammirato (1983) states that somatic embryogenesis has been sufficiently observed in enough species and families to demonstrate that it is not a phenomenon restricted to just a few taxa. He also states that the possibility exists that cells from any plant, given the appropriate stimuli and conditions, could be fostered to embark on the embryonic pathway.

7. Clonal propagation through tissue culture techniques

Morel (1960) was the pioneer in applying shoot-tip culture as a tool of clonal multiplication. Orchids were the first plants to be propagated by tissue culture. He also described the elimination of internal virus in the orchid Cymbidium. His successes in clonal multiplication and elimination of internal virus reported in two papers (Morel 1960,1965) subsequently provided momentum for in vitro clonal multiplication. Murashige and Skoog (1962) published a revised medium for rapid growth of tobacco tissue cultures. This medium has probably been used more often than any other for a wide range of plant species. Murashige should be credited as the dominant figure in the establishment of micropropagation

techniques. He developed a sequence of 3 developmental stages (Murashige 1974). These stages are: Stage I, the establishment of the aseptic culture, Stage II, multiplication of propagula, and Stage III, preparation for re-establishment of plants in soil.

Rapid clonal propagation using *in vitro* techniques for asexual propagation is, for commercial purposes, the most advanced area of tissue culture. Hussey (1978) and de Fossard (1976) have listed the following reasons for efficient and reliable vegetative propagation which may utilize plant tissue culture techniques: 1) rapid multiplication of new hybrid cultivars in many species; 2) elimination of virus, fungus, and bacteria from infected stocks; 3) propagation of genetically uniform parent plants; 4) large-scale production of hybrid seeds; 5) year-round propagation of clones. The highly favorable characteristics of clonal propagation *in vitro* have naturally attracted the attention of horticulturists, foresters, and plant breeders. Numerous species have been tissue-cultured and were listed by Murashige (1974), Vasil *et al.* (1979), Hughes (1982), and Hu and Wang (1983).

Many species such as ferns, foliage plants, woody ornamentals, bulbs, herbaceous ornamentals, and other crops are being produced by several commercial laboratories in the U.S.A., France, West Germany, Taiwan, and elsewhere (George and Sherrington 1984). But, all species in which controlled organogenesis can be induced *in vitro* may not be suitable for large-scale clonal propagation because the whole process may be too expensive, the rate of multiplication may be too slow, there may be high mortality of plantlets when explanted to soil, or induced genetic

variation may occur in the plantlets through aneuploidy or polyploidy during cell proliferation *in vitro* (Murashige 1977b and George and Sherrington 1984). Currently, most commercial laboratories use shoot-tip culture and induced axillary branching to overcome the last-mentioned problem (Murashige 1974).

B. Factors Affecting Shoot-tip and Meristem Cultures

1. Explant size and physiological age

The sizes of meristem tips are one of the crucial factors which govern the regenerative capacity of meristems and also increase the probability of recovering pathogen-free plants. Murashige (1974) indicated that certain terms or concepts have been grossly misused. Foremost among these are "meristem culturing", "meristemming", and "mericlones." According to his definitions, the explant for shoot apical meristem culture is restricted to the terminal dome and measures less than 0.1 mm in height. True meristem culture signifies an explant whose dimensions are much too small to be of practical value in rapid clonal propagation. According to Hu and Wang (1983), the explant for meristem culture may either be the apical dome (apical meristem) or, more frequently, apical dome plus a few subjacent leaf primordia. And, the shoot-tip explants are usually taken from the tender tip of the growing shoot measuring 2 cm or less in length. Styer and Chin (1983) stated that all explants from shoot tips differ in size and in the number of leaf primordia, and apical meristems without any leaf primordia have the highest probability of producing plants lacking detectable levels of

viruses, and thus, they are the explants most often used for the production of disease-indexed plants.

As a general rule, very small explants have low survival rates in culture and actual sizes of the explants depend upon the species. Wang and Ma (1978) reported that Chrysanthemum shoot tips between 0.2 and 0.5 mm and shoot meristems of 0.1 and 0.2 mm produced only a single shoot but larger explants, 0.5-1.55 mm in length, produced multiple shoots, demonstrating that number of shoots produced from a shoot-tip explant may also be influenced by the size of the explant. According to Hughes (1982) explant size is rarely a problem unless the purpose of the culture system is to obtain virus-free plants. At one time, the apical meristem area was believed to be virus-free, but more recent studies have demonstrated that viruses may be found even in the meristematic area (Hughes 1982). Generally, the larger the size of the shoot tip, the greater will be the number of shoots generated. But, if the experiments are aimed at eliminating viral pathogens, the reverse is the rule; i.e., the smaller the size of the shoot tip, the greater will be the chance of eliminating the virus (Kartha 1981).

2. Culture medium

Murashige and Skoog (1962) reported the mineral nutrient requirements and a revised medium for rapid *in vitro* growth and high yield of tobacco callus tissue. This revised medium (MS) has also been very satisfactory for culturing shoot tips and meristems from ornamental species. Linsmaier and Skoog (1965) also reported the organic growth factor requirements in

tobacco tissue cultures. This LS medium also has been one of the most popular media used in ornamental plant tissue cultures. Gamborg et al. (1968) reported the nutrient requirements of suspension cultures from soybean root cells, now called B5 medium. They found that the cells required thiamine, 2,4-D, and ammonium nitrogen in addition to the usual mineral salts and sucrose. MS, LS, and B5 media are all high N, P, and K salt media. Sometimes, roots are unable to be initiated in such high salt concentrations, regardless of the types of hormones present. When the salt concentration in the medium is lowered to one-half, one-third, or one-fourth of the standard strength, rooting often becomes abundant (Kartha and Constabel 1974, Lane 1979, Skirvin and Chu 1979, Hasegawa 1980, Kartha et al. 1981, Anderson 1984, and Khosh-khui et al. 1984).

Murashige and Skoog (1962) used an EDTA chelated form of iron to bypass the precipitation of iron. Nitsch (1969) demonstrated that iron was required to form plantlets from microspores of tobacco plants, and the chelated form was more effective than ferric citrate. In 1970, Steiner and van Winden developed techniques for preparation of iron chelate that did not precipitate, thus making iron more available to plant tissue cultures.

Two excellent reviews by Huang and Murashige (1977) and Gamborg et al. (1976) summarized various media and their components. Gamborg et al. decided that successful plant tissue culture depends on the choice of nutrient media and the cells of most plant species can be grown on completely defined media. They also pointed out that cells generally have no essential requirement for complex organic supplements such as amino

acids, casein hydrolysate, yeast extract or coconut milk. Styer and Chin (1983) stated that the most common organic supplements included in meristem and shoot-tip culture media are the vitamins such as thiamine, nicotinic acid, pyridoxine, the amino acid glycine, and myo-inositol. A more complete vitamin formulation has been developed by Staba (Miller et al. 1982). Undefined organic amendments, e.g., coconut milk and activated charcoal, have been used as medium supplements to enhance shoot or root formation (Snir and Erez 1980, and Rosati et al. 1980, and Kerbauy 1984). Where the nutritional requirements for a given taxon have not been established, vitamins are often added to the medium as a precautionary measure (Hughes 1982).

It is often necessary to revise the culture medium for successful shoot-tip and meristem cultures because the nutritional requirements are species-dependent. For example, Anderson (1984) revised the Murashige and Skoog 1962 organic formula for successful shoot multiplication of Rhododendron cultivars because cultures on MS medium exhibited a toxic reaction. He reduced the concentrations of nitrogen, potassium, phosphorus, and iodine, while iron concentration was doubled to 200 uM. These changes reduced the salinity of the inorganics from 5.2 to 2.2 mmho cm^{-1}. As a result, he achieved very successful shoot multiplication as well as rooting of Rhododendron cultivars.

3. Growth regulators

The application of plant growth regulators has been another important event in the clonal propagation of ornamental species. The balance of

endogenous and exogenous growth regulators controls the initiation and development of shoots, roots, plantlets, and even callus. Skoog and Miller (1957) were the pioneers in applying growth regulators to plant tissue cultures. They found that auxin/cytokinin ratios within the concentrations tested provided a common mechanism for the regulation of all types of growth. This finding has greatly influenced the *in vitro* clonal propagation of ornamental and other species. While there is no generalized ratio for shoot or root induction, both an auxin and a cytokinin are usually added to the medium in order to obtain morphogenesis (Hill 1967, Kaul and Sabharwal 1972, Rao *et al*. 1973, Ziv 1979, Walkey *et al*. 1980, Kartha *et al*. 1981, Smith and Murashige 1982, Lineberger 1983, Stephens *et al*. 1985, and Tripathi and Britaillon 1985). Lam and Street (1977) reported that, for an auxin source, CPA (4-chlorophenoxy acetic acid) was less effective in maintaining a high growth rate of cells than 2,4-D (2,4-dichlorophenoxy acetic acid) or 2,4,5-T (2,4,5-trichlorophenoxy acetic acid). Halperin (1966) and Hill (1967) found that the high levels of auxin, particularly 2,4-D, suppressed plant morphogenesis in *Convolvulus arvensis* and carrot. Engvild (1978) also reported very similar suppression in tobacco plants. Kaul and Sabharwal (1972) reported that root and shoot differentiation was obtained by providing appropriate amounts of auxin and cytokinin in the culture medium and morphogenetic responses of explants were not species specific in *Haworthia*. Fonnesbech and Fonnesbech (1980) reported that the highest number of shoots was obtained with the combination of 10 mg/l PBA (benzylamino-9-(2-tetrahydropyranyl)-9H-purine) and 2 mg/l IAA (indoleacetic acid), rooting

of shoots was best on MS basal medium supplemented with 2 mg/l IAA, and the morphology of the in vitro propagated plants was similar to the original clones. Auxins such as NAA, IAA, IBA, and 2,4-D, etc. are usually reported to cause shoot induction in Monstera (Fonnesbech and Fonnesbech 1980), rose (Hasegawa 1980), Gypsophila (Kusey et al. 1980), Bougainvillea (Sharma et al. 1981), Grevillea (Ben-Jaacov and Dax 1981), Rosaceous plants (Norton and Boe 1982), cherry (Lineberger 1983), Yucca (Pierik and Steegmans 1983), myrtle (Khosh-khui et al. 1984), Stevia (Tamura et al. 1984), and Hedychium (Tripathi and Bitaillon 1985).

Among cytokinins, BA was the most effective for multiple shoot formation by shoot-tip or meristem cultures of Gypsophila (Kusey et al. 1980), rose (Hasegawa 1980), Hosta decorata (Papachatzi et al. 1981), several other rosaceous plants (Norton and Boe 1982), Lythrum (Heuser 1983), Yucca (Pierik and Steegmans 1983), and Myrtle (Khosh-Khui et al. 1984). However, Fordham and Stimart (1982) reported that BA was the least effective for multiple shoot formation in azaleas, and shoot proliferation was affected by genotype. Many plant tissues have an absolute cytokinin requirement but others are cytokinin independent. For example, Phaseolus lunatus is cytokinin dependent (Mok 1979), while Nicotiana tabacum is cytokinin independent (Meins and Binns 1979). Thus, cytokinin requirements of plant cells and tissues in culture may be both genetic and epigenetic (Hughes 1982). Appropriate auxin and cytokinin levels must be determined for each species and variety under study. Even different sources within the same plant may vary in their auxin and cytokinin requirements.

4. Environmental conditions

The culture environment includes all factors other than culture medium. Even though so many factors can potentially affect shoot-tip and meristem cultures, the most important factors are light and temperature. The relative humidity of the culture facility is usually not important since the relative humidity of the microenvironment within a culture is about 100% (Murashige 1974).

a. Light The light requirements of tissue cultures are not the same as those of autotrophically developing plants since carbohydrate is adequately provided. However, several studies have shown that light plays very important roles to regulate certain morphogenetic or organogenetic processes (Bajaj and Pierik 1974, Hussey 1976, and Kato 1978a,b).

The three characteristics of light that affect plant tissue cultures are: light intensity, photoperiod, and light quality. Morphogenetic and organogenetic requirements for light in tissue cultures may be satisfied by one or more of these factors.

1) Light intensity In an experiment with excised Gloxinia shoot tips, Hamaraki (1971) reported that cultures growing without light were etiolated, the leaf blades were small and yellowish green, and there was a larger amount of tuber-like callus. The plant and leaf size increased with up to 300 ft-c (3226 lux) of light, and leaf color darkened with an increase in light intensity. Hasegawa et al. (1973) found that 1,000 lux was the optimum light intensity for spear as well as root initiation in asparagus shoot-apex culture. An intensity of 10,000 lux was clearly excessive; no new spears resulted. Similar results were

observed in tropical foliage plants (Miller and Murashige 1976). However, a light intensity of 3,000 lux was about optimum for the multiplication of shoots in recultures (subcultures of shoots) of Dracaena, Scindapsus and Syngonium, and Cordyline showed maximum shoot increase under a light intensity of 300 lux (Miller and Murashige 1976). In cultures of Gerbera and many other herbaceous genera, the optimum light intensity for the initiation of shoots is 1,000 lux, 300 lux being inadequate, and light intensity higher than 3,000 lux being strongly inhibitory (Murashige 1977a). The optimum light intensity sometimes appears to depend on the daylength used (Hussey 1978). It was also reported that axillary shoot multiplication was stimulated by PAR (photosynthetically active radiation), with either 70 $uEm^{-2}s^{-1}$ (3591 lux) or 130 $uEm^{-2}s^{-1}$ (6669 lux) resulting in the highest number of shoots in Hosta decorata 'Thomas Hogg', using cultured shoot tips. However, shoots maintained under 2.5 $uEm^{-2}s^{-1}$ were stunted and contained little chlorophyll; and those shoots under 130 $uEm^{-2}s^{-1}$ had leaf tips which were necrotic. Maximum growth occurred under 70 $uEm^{-2}s^{-1}$ (approx. 3591 lux) (Papachatzi et al. 1981). More shoots were produced at a light intensity of 65 $uEm^{-2}s^{-1}$ than 41 $uEm^{-2}s^{-1}$, but light intensity had no apparent effect on shoot length for in vitro propagation of Deutzia x lemoinei var. compacta (Hildebrandt and Harney 1984). Heuser (1983) found no differences in axillary shoot number among the 3 irradiance levels of 15, 6, and 1.5 $uEm^{-2}s^{-1}$. However, dark incubation allowed more rapid formation of root initials in many rosaceous plants, and considerable callus growth resulted in most species under continuous darkness (Norton and Boe 1982).

In general, a wide range of light intensities is suitable for various cultures. Intensities below 1,000 lux are used only occasionally, while intensities between 1,000 and 4,000 lux are the most common (Styer and Chin 1983). Light intensity has been shown to affect the type of growth in culture. Increased intensity from 3,000 to 6,000 lux caused an increase in shoot formation in one cultivar of Begonia x hiemalis, but no increase in dry weight (Hughes 1982). Higher light intensities, e.g. 10,000 lux were required before being transferred to soil to enhance their ability to survive in the greenhouse (Murashige 1974, Hughes 1982, Styer and Chin 1983, and Hasegawa et al. 1973).

2) Photoperiod Like light intensity, the effective photoperiod varies with most plants growing in vitro. Obviously, the key is the total radiant energy of specified quality to which the culture is exposed rather than the length itself that the culture is exposed to light (Murashige 1974). Moreover, the combined effect of both light intensity and photoperiod plays more important roles for manipulating certain morphogenetic or organogenetic processes. It is reasonable to expect varying optima in the length of the daily exposure to light for a given species, depending on the light intensity used (Murashige 1974).

Haramaki (1971) reported that cultures of Gloxinia under no light were etiolated and cultures with 8 hr or less of light still showed some signs of etiolation. In clonal multiplication of Gerbera through tissue culture, Murashige et al. (1974) mentioned that photoperiods of 12 to 16 hrs provided the most favorable length of daily exposure for shoot division and shoot quality. For the initiation of shoots and roots from

asparagus shoot-apex cultures, the length of the optimum daily exposure to light was somewhere in the range of 4-20 hrs (Hasegawa *et al.* 1973). In the shoot apical-meristem culture of *Pharbitis nil*, Bapat and Rao (1977) found that the growth effect of different photoperiods showed striking differences. Long photoperiods were suitable for leaf expansion and chlorophyll formation but produced leaves with short petioles. Etiolated plantlets were produced from meristems in complete dark. Similar results had also been already obtained with *Gerbera* and *Gloxinia* by Murashige *et al.* (1974) and Haramaki (1971), respectively.

According to Murashige (1977a), continuous illumination does not allow maximum organogenesis to occur. In most cases, the optimum photoperiod has been 16 hr. Continuous illumination in tobacco callus culture was distinctly unfavorable, and shoot initiation was repressed significantly. He also stated that the best photoperiod for a given tissue culture is dependent upon the light used, and probably other factors. Many workers have commomly used 14 to 18 hr photoperiods for tissue cultures (Styer and Chin 1983, Dabiski *et al.* 1979, Kerbauy 1984, Stoltz 1984, Blazich and Novitzky 1984, Stephens *et al.* 1985, Ettinger and Preece 1985, and Economou and Spanoudaki 1985).

Photoperiod response in culture may be influenced by changes in endogenous levels of auxin and cytokinin. Some researchers (Heide 1965 and Heide and Skoog 1967) have demonstrated that photoperiods influenced the endogenous auxin and cytokinin levels in *Begonia* and *Bryophyllum*. For *in vitro* propagation of ornamental rosaceous plants, Norton and Boe (1982) demonstrated that when explants were incubated in continuous dark, roots

were short. They suggested that this may be caused by a high endogenous auxin concentration in shoots which inhibits root elongation. Endogenous auxin is degraded in light and this could result in a lower total auxin concentration in the high light-treated than in the dark-treated explant.

3) Light quality Spectral quality of light can be very important in regulating various morphogenetic effects (Hussey 1978). The quality of light, particularly spectrum or wavelength, can be manipulated by artificial illumination. For use with tissue culture, the lamp should contain adequate doses of blue and red light, since both root initiation and shoot formation are promoted by these wavelength (Murashige 1977a). Other spectral regions for plant tissue cultures should not be ignored in selecting the lamp. Grolux fluorescent lamps (Haramaki 1971, Hasegawa et al. 1973, Murashige et al. 1974, Murashige 1977a, Lineberger and Wanstreet 1983, and Smith and Murashige 1982) and Cool White fluorescent lamps (Hasegawa et al. 1973, Murashige 1977a, Papachatzi et al. 1981, Norton and Boe 1982, Heuser 1983, Economou and Spanoudaki 1985, Ettinger and Preece 1985, Tripathi and Bitaillon 1985, and Stoltz 1984) have been commonly used for plant tissue cultures because fluorescent lamps provide a high output of photosynthetically active radiation (PAR) between 400 and 700 nm and photomorphogenetic radiation between 700 and 850 nm (McFarlane 1978 and Blazich and Novitzky 1984). The spectrum from the lamps generally matches the requirements of plants. Lately, Cool White fluorescent lamps have been used more often than Grolux lamps even though Hasegawa et al. (1973) reported that there was no difference in effectiveness between the two lamps on asparagus spear and root initiation in vitro.

The photosynthetic reaction is driven by light in the spectral region between 400 and 700 nm. Most photostimulus reactions are controlled by wavelengths either in the blue region (400-460nm) or in the red and far-red regions between 650 and 800 nm. The latter reactions between 650 and 800 nm have been found to be controlled or partially controlled by the phytochrome pigments (McFarlane 1978). Seibert (1973) reported that blue light in the region of 467 nm was effective in inducing shoots in tobacco callus. Purple light was also effective. These experiments demonstrate that photomorphogenesis does occur *in vitro*, possibly by interacting with phytochrome pigments and other pigments. Murashige (1974) also reported that key organogenetic processes in tissue cultures are photomorphogenic phenomena, most probably regulated by phytochrome.

b. *Temperature* Few studies have been carried out to find optimum incubation temperatures. The optimum incubation temperature varies with plant species and phenomena being considered. Most researchers studying meristem and shoot-tip cultures have selected a constant temperature between 20 and 28 ^{o}C, with the great majority in the middle of this range, i.e., 24-26 ^{o}C (Hu and Wang 1983).

Hasegawa *et al.* (1973) reported that a diurnal fluctuation in temperature might be desirable for maximum shoot formation in asparagus shoot-apex cultures. No difference at different temperatures of 27, 22, 17, and 12 ^{o}C was observed in the rate of spear formation. However, root initiation was reduced markedly and progressively with each decrease in the night temperature. A constant 27 ^{o}C was the optimum temperature for the multiplication of shoots and roots and for the development of

transplantable plants. The diurnal temperature fluctuation may be desirable for some plants, especially for those adapted to temperate and desert climates (Murashige 1974 and 1977a). Duron (1984) employed 24 °C day and 17 °C night temperature for in vitro propagation of ornamental Malus x perpetu 'Evereste'. However, most workers who used a fluctuating diurnal temperature did so through arbitrary choice, apparently without experimental support (Hu and Wang 1983).

Practically speaking, the incubation temperatures in plant tissue cultures have been usually held constant. It was reported that the optimum temperature for both differentiation and growth of Lilium bulb scales was 20 °C in the range between 20 and 30 °C (Takayama and Misawa 1979). Papachatzi et al. (1981) suggested that the optimum temperature depended upon the light intensity used, while higher or lower temperatures than 21 °C resulted in a reduction in the number of adventitious shoots of Hosta decorata 'Thomas Hogg'. Pierik and Steegmans (1983) stated that for vegetative propagation of Yucca elephantipes, the number of laterals per shoot increased from 6.0 to 7.6 when temperature was increased from 20 to 25 °C. A further increase from 25 to 29 °C had no significant effect on the number of axillary shoots.

Constant incubation temperatures between 24 and 26 °C are common for shoot-tip and meristem cultures for most families although diurnal temperature fluctuations have been employed for some species (Styer and Chin 1983). Different incubation temperatures for shoot and root formation have been reported occasionally (Ben-Jaacov and Dax 1981). Recently, most researchers have employed a constant temperature of 24-26

^{o}C to maintain the cultures of most plant species (Kerbauy 1984, Stoltz 1984, Tamura et al. 1984, and Stephens et al. 1985).

5. Seasonal fluctuation

Seasonal fluctuations are known to affect the morphogenetic potential of stock plants, even after shoot-tip and meristems are cultured (Hughes 1982). Because of their strong growth potential and their low virus concentration, actively growing shoot tips are usually recommended for meristem and shoot-tip cultures (Hu and Wang 1983). It was reported that carnation meristems survived better when excised from plants during the actively growing season of early spring and early autumn, compared to those taken during summer and winter (Stones 1963). For plant species with a definite dormant period such as bulbs and corms, the best results are probably expected at the end of their dormancy period (Karatha 1981 and Hu and Wang 1983). Stephens et al. (1985) stated that reproductive stem tips rooted more slowly than vegetative stem tips in Impatiens species. Morphogenetic ability seems to be enhanced in periods of vegetative growth, for a wide range of species (Hughes 1982).

6. Surface disinfection

Surface disinfestation is one of the most important procedures for conducting successful experiments or propagating plants commercially through shoot-tip and meristem cultures since contamination has often been very critical in tissue-culture research, shoot-tip and meristem culture, and even in commercial micropropagation.

The most common surface disinfectant is sodium hypochlorite (NaOCl) which is often 5-6% of the active ingredient in commercial bleach and is usually used as a 10-15 % dilution of commercial bleach (Hu and Wang 1983). However, both the concentration and the soaking duration depend upon the plant materials used. The concentration and duration have to be adjusted according to need. The common soaking duration is 10-15 minutes. The higher concentration or longer duration results in tissue damage or cell death. Other commonly used surface disinfectants include calcium hypochlorite and mercury chloride. The concentration and the duration are very similar to that of sodium hypochlorite. After soaking, 3-4 rinses with sterile distilled water are necessary to remove the disinfectant before the final excision of the explants. A concentration of 70-75% ethyl alcohol (EtOH) is also often used as a surface disinfectant.

The most common contaminants are virus, bacteria, and fungi (Hakkaart and Hartel 1979). Langhans et al. (1977) found that tissue cultured plants were infected with several viruses in Chrysanthemum through the leafy callus stage and back to plantlets. Host plant callus tissue derived from shoot tips would seem to be a good place to store viruses rather than in greenhouse-grown stock plants. However, it was reported that dahlia mosaic virus was eliminated from Dahlia pinnata by culture of 0.2-1.0 mm meristem tips (Mullin and Schlegel 1978). Hakkaart and Hartel (1979) reported successful virus eradication from some Pelargonium zonale cultivars by meristem-tip culture. A similar result was reported for Passiflora caerulea by Hakkaart and Versluys (1981). Debergh and Maene (1981) reported that bacteria were the most serious contamination in

tissue culture of numerous ornamental species, and they could not find plants that would yield non-contaminated explants after the usual sterilization procedure in most species. But, Knauss (1976) described the indexing procedure to get Dieffenbachia picta free of fungus and bacteria. He obtained fungi- and bacteria-free plants by his indexing prodedure. Murashige (1974) warned that plants obtained from shoot-tip and meristem cultures should not be assumed to be disease- or pathogen-free because the claim of a disease-free state must specify the pathogens which have been excluded, and the evidence must have been obtained through systematic tests. Indexed plants should therefore specify the disease(s) that they have been indexed for.

C. Ornamental Species for which Shoot-tip and Meristem Cultures have been Successful during the Past Decade

Species	Explant	Morphogenic Response	Author
Ajuga reptans	shoot tip	multiple shoots	Lineberger and Wanstreet (1983)
Anigozanthus hybrida	apical shoot, sprouting, rhizome, and dormant axillary bud	multiple shoots	McComb and Newton (1981)
Anigozanthus sp.	apical shoot, sprouting, rhizome, and dormant axillary bud	multiple shoots	McComb and Newton (1981)
Bougainvillea glabra	shoot tip	multiple shoots	Chaturvedi et al. (1978)

Bougainvillea glabra	shoot apex	multiple shoots and roots	Sharma et al. (1981)
Buddleia davidii	meristem	virus-free plants	Duron and Morand (1978)
Chaenomeles japonica	shoot tip	multiple shoots and roots	Norton and Boe (1982)
Chrysanthemum cinerariaefolium	shoot tip	multiple shoots	Grewal and Sharma (1978)
Coleus blumei	meristem	multiple shoots and roots	Smith and Murashige (1982)
Cordyline terminalis	stem tip	adventitious shoots	Miller and Murashige (1976)
Cotoneaster dammeri	shoot tip	multiple shoots and roots	Norton and Boe (1982)
Crataegus brachyacantha	shoot tip	multiple shoots and roots	Norton and Boe (1982)
Dahlia pinnata	shoot tip	virus-free plants	Mullin and Schlegel (1978)
Deutzia x lemoinei	shoot tip	multiple shoots	Hildebrandt and Harney (1984)
Dianthus caryophyllus	frozen shoot tip	shoots	Seibert (1976)
	nodal stem	multiple shoots	Roest and Bokelmann (1981)
	meristem	multiple shoots	Leshem (1983a,b) Ziv et al. (1983)
Dianthus sp.	shoot tip	shoots	Gukasyan et al. (1977)
Dieffenbachia picta	shoot tip	shoot	Knauss (1976)
Dracaena godseffiana	shoot tip	multiple shoots	Miller and Murashige (1976)
Easter Cactus	shoot tip	adventitious shoots	Koenigsberg and Langhans (1976)
Episcia sp.	stem tip	adventitious shoots	Bilkey and McCown (1979)

Forsythia sp.	meristem	virus-free stem	Gautheret (1977)
Fuchsia sp.	shoot tip	multiple shoots	Dabin and Choisez-Givron (1982)
Gardenia jasminoides	shoot tip	multiple shoot and roots	Economou and Spanoudaki (1985)
Grevillea rosmarinifolia	shoot-segment	multiple shoots	Ben-Jaacov and Dax (1981)
Gypsophila paniculata	shoot tip	multiple shoots, calli, and roots	Kusey *et al.* (1980)
Hedychium roxburghii	meristem	shoots, roots, and buds	Tripathi and Bitaillon (1985)
Hippeastrum hybridum	meristematic areas	adventitious shoots	Hussey (1978)
Hosta decorata	shoot tip shoot tip	multiple shoots axillary shoots	Allen (1976) Papachatzi *et al.* (1981)
Hosta fortunei	meristem	multiple shoots	Dick (1980)
Hydrangea macrophylla	bud	multiple shoots and roots	Stoltz (1984)
	stem tip	multiple shoots	Bailey *et al.* (1985)
Impatiens sp. 'Pikinini'	meristem	single shoot	Koenigsberg and Langhans (1976)
Impatiens sp.	shoot tip	multiple shoots and roots	Stephens *et al.* (1985)
Kalmia latifolia	shoot tip	axillary shoots	Lloyd and McCown (1980)
Lythrum virgatum	shoot tip	multiple shoots	Heuser (1983)
Macropidia fuliginosa	apical-shoot	multiple shoots	McComb and Newton (1981)
Monstera deliciosa	shoot tip	multiple shoots and roots	Fonnesbech and Fonnesbech (1980)

Myrtus communis	shoot tip	multiple shoots and roots	Khosh-Khui *et al.* (1984)
Narcissus sp.	meristematic areas	adventitious shoots	Hussey (1978)
Nerine sp.	meristematic areas	adventitious shoots	Hussey (1978)
Pelargonium sp.	meristem	adventitious shoots	Cassells *et al.* (1982)
Pelargonium zonale	meristem	virus-free shoots	Hakkaart and Hartel(1979)
Petunia hybrida	meristem	shoot buds	Sharma and Mitra (1976)
Pharbitis nil	meristem	roots and shoots	Bapat and Rao (1977)
	shoot tip	floral primordia	Templeton and Cline (1979)
Potentilla sp.	shoot tip	multiple shoots and roots	Norton and Boe (1982)
Prunus hybrida	shoot tip	multiple shoots, roots and callus	Lineberger (1983)
Prunus sp.	shoot tip	multiple shoots and roots	Norton and Boe (1982)
Pyracantha coccines	shoot tip	multiple shoots and roots	Norton and Boe (1982)
Rhododendron hybrida	shoot	multiple shoots	McCown and Lloyd (1983)
	shoot	multiple shoots	Anderson (1984)
	shoot tip	multiple shoots and roots	Ettinger and Preece (1985)
Rhododendron sp.	shoot tip	axillary shoots	Anderson (1978)
	shoot tip	adventitious shoots	Fordham and Stimart (1982)
	shoot	multiple shoots	McCown and Lloyd (1983)
Rosa hybrida	shoot tip	multiple shoots	Hasegawa (1979)
	shoot	rooted shoots	Skirvin and Chu

			(1979)
	shoot tip	multiple shoots	Hasegawa (1980)
Rosa indica	shoot apex	multiple shoots	Avramis et al. (1982)
Ruscus hypophyllum	shoot tip and inflorescence	axillary shoots and adventitious shoots	Ziv (1983)
Sansevieria trifasciata	leaf	multiple shoot and roots	Blazich and Novitzky (1984)
Spathiphyllum clevelandii	shoot tip	rooted shoots	Strode and Oglesby (1978)
Stevia rebaudiana	stem tip	multiple shoots	Tamura et al. (1984)
Syngonium podophyllum	shoot tip	multiple shoots	Miller and Murashige (1976)
	shoot tip	multiple shoots	Makino and Makino (1978)
Syringa vulgaris	shoot tip	multiple shoots	Hildebrandt and Harney (1983)
Torenia fournieri	stem-segment	adventitious buds and floral buds	Tanimoto and Harada (1981)
Yucca elephantipes	shoot tip	axillary shoots and roots	Pierik and Steegmans (1983)
Yucca glauca	shoot tip and rhizome	multiple shoots	Bentz and Parliman (1985)
Veronica sp.	shoot tip	axillary shoots	Stapfer et al. (1985)

III. OBJECTIVES

Experiments I and II were conducted to determine the effects of 3 cytokinins (kinetin, BA, and 2iP) and an auxin (NAA) on *in vitro* propagation of *Impatiens* sp. 'T63-1' and 'Starfire' and to find what kind and concentration of cytokinins were best for multiple shoot production by shoot-tip cultures. Additionally, these studies were conducted to establish a rapid propagation method for 2 Indonesian *Impatiens* interspecific hybrids.

Experiment III was conducted to confirm the best type and concentration of cytokinins for shoot multiplication on the basis of the results of experiments I and II and to find whether or not cytokinin requirements of two cultivars were genotype-dependent for shoot multiplication.

IV. MATERIALS AND METHODS

A. Stock Plant Culture

Clones of Impatiens sp. 'T63-1' and 'Starfire' were grown in the Iowa State University (ISU) Department of Horticulture research glasshouse from rooted cuttings. 'T63-1' is a hybrid of a cross between a Java and New Guinea Impatiens and part of the ISU Impatiens breeding collection. 'Starfire' is a hybrid of a cross between a Celebes and New Guinea Impatiens and a cultivar released by ISU. All plants of each clone were fertilized once a week with 100 ppm N of a 20-20-20 analysis water-soluble fertilizer.

Vigorously growing stem tips were collected from plants in the research glasshouse. They were rinsed in cold tap water and expanded leaves were cut off. The tips were trimmed to a length of 1-2 cm and put in a side-arm flask containing 0.5% NaOCl (10% v/v commercial laundry bleach). The flask was aspirated for 15 min. and swirled continuously, after which all steps were performed aseptically in a laminar-flow transfer hood. After 4 rinses in sterile, deionized water, all leaves and visible leaf primordia were trimmed from each shoot, and it was cut to a length of 2-3 mm, from cut base to apical meristem. The prepared explant was plated onto 10 ml of semisolid MS basal medium (Murashige and Skoog 1962), cut base down, in 25 x 150 mm glass culture tubes. Each tube was capped with a plastic Kaput (closure) (Bellco Glass, Inc.) and sealed with a Celon (cellulosic band) (Thatcher Plastic, Inc.).

MS basal medium was used to produce stock plants in vitro. No plant

growth regulators were added to the medium and the original growth regulators, kinetin and IAA, were deleted (Murashige and Skoog 1962). The pH of the medium was adjusted to 5.75-5.85 before adding 8 g/liter Difco Bacto-agar. The medium was autoclaved 10 min. at 1.1 kg cm^{-2} and 121 ^{o}C to dissolve agar, and 10 ml of medium was dispensed into glass tubes with an automatic dispenser. Each tube was capped with a plastic Kaput and autoclaved 15 min. as described above.

The cultures were incubated in a growth chamber at 26 ^{o}C under 16 hours of illumination of approx. 120 uE $m^{-2}sec^{-1}$ with Cool White fluorescent lamps (General Electric, Inc.). Photosynthetically active radiation (PAR) was measured at the top shelf of the growth chamber with a LiCor Li-195 Quantum/Radiometer/Photometer (LiCor Instruments Corp., Lincoln, Neb.) equipped with a Li-1905 Quantum sensor. Pathogen-free cultures were continuously selected and incubated in the same growth chamber until the cultures were large enough to be subcultured. All selected cultures with 5-8 cm long stems were aseptically "pinched" to cause axillary shoot growth and these shoots were steadily subcultured to get enough pathogen-free plant materials.

B. Experiments I and II

Combinations of NAA and one of 3 cytokinins were added to the basal medium. All treatment combinations of NAA and 3 cytokinins are shown in table 4-1. The pH was adjusted to 5.75-5.85 with 0.1 N KOH and 0.1 N HCl before adding 8 g/liter Difco Bacto-agar. The autoclaving process was as mentioned above for the MS basal medium. Then, the culture medium was

stored at 4 ^{o}C in the refrigerator for 1-4 weeks.

The cultures were grown *in vitro* for 4 weeks after being subcultured as stock plants for use in experiments I and II. It was not necessary to sterilize the plant materials before plating the explant onto the culture medium because the selected stock-plants were apparently pathogen-free. The size of the explant was similar to glasshouse plant materials used for stock plant culture. All other procedures were also similar to the stock-plant culture. All steps were performed under a laminar-flow transfer hood. The cultures under experimentation were incubated at the top self in the same growth chamber as the stock cultures. Environmental conditions such as illumination, temperature, and air-flow were similar to those for stock plant culture. Data were taken after 3 and 6 weeks of incubation. The number of shoots and roots were counted at 3 and 6 weeks, respectively. The total length of shoots and the diameter of explants were also measured at 6 weeks.

Experiments I and II were designed as a Randomized Complete Block design (RCBD). Each experiment was composed of 60 treatments, and each treatment consisted of 5 replications. The experiments were 'blocked' over time. F-tests and regression analyses were applied to the data from experiments I and II. Other statistical analyses necessary to interpret the data were also applied.

C. Experiment III

The basal medium was the same as that used for stock plant culture. Two different cytokinins, kinetin and 2iP, were added to the basal medium.

The cytokinin concentrations used for experiment III were 0, 20, 40, and 60 uM. These concentrations were decided upon from the results of experiments I and II. The pH adjustment of the medium and the autoclaving process were similar to those of experiments I and II. The storage conditions of the culture medium was also similar to that of experiments I and II.

The 2 genotypes used for experiment III were 'T63-1' and 'Starfire'. Shoot-tip explants were obtained from shoot cultures of the genotypes grown in vitro for 4 weeks. All other steps and procedures were similar to experiments I and II. Environmental conditions for incubation were also similar except temperature range of 26-28 ^{o}C. The number of shoots were counted, and shoot length per shoot and explant diameter were all measured after 6 weeks in culture.

Experiment III was designed as a Randomized Complete Block design (RCBD). The experiment consisted of 16 treatments (8 treatments for 'T63-1' and 8 for 'Starfire'), and each treatment consisted of 9 replications. However, only 5 replications among 9 were used for data analyses since 4 replications were badly affected by high temperature (31 ^{o}C) on one side of the growth chamber. Explants in replications under high temperature did not grow at all or died, while explant on the other side (26-28 ^{o}C) grew normally. The experiment was originally 'blocked' over space and time but all replications were grouped for structured analysis. F-tests and regression analyses were applied to data from experiment III. Other statistical analyses were necessary to interpret the data such as means, LSD tests, etc.

Table 4-1. Treatments of NAA and 3 cytokinins over 5 cytokinin levels for Experiments I[a] and II[b]

		Kinetin					BA					2iP				
unit : uM		0	10	20	40	80	0	10	20	40	80	0	10	20	40	80
NAA(uM)	0	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	4	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	6	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

[a] Impatiens sp. 'T63-1'.

[b] Impatiens sp. 'Starfire'.

[x] Treatment combinations of NAA and each cytokinin.

V. RESULTS

A. Experiment I: 'T63-1'

Shoot multiplication of Impatiens sp. 'T63-1' showed significant differences for cytokinin type and cytokinin level (Table 5-1). BA was most effective for shoot production, and kinetin was least effective (Figure 5-1). Shoot multiplication was enhanced by cytokinin concentrations from 10 to 40 uM (Table 5-2). The highest shoot numbers were proliferated at 20 uM of cytokinins. Only 1-2 shoots were proliferated with no cytokinin. The interactions of cytokinin type * cytokinin level and cytokinin type * NAA also produced significant differences for shoot numbers (Table 5-1). Each cytokinin affected shoot multiplication differently, depending on concentration. Compared to other cytokinins, BA enhanced shoot multiplication with an optimal concentration of 10 uM and higher concentrations inhibited shoot multiplication (Fig. 5-1). For 2iP, shoot multiplication was highest at 40 uM, but fewer shoots were produced than with BA. Kinetin caused to proliferation of the fewest shoots of any cytokinin, and the highest kinetin concentration produced the highest shoot numbers. The effect of cytokinin type was also dependent on NAA concentration (Fig. 5-2). BA produced the highest shoot numbers at 2 uM NAA, and lower shoot numbers for lower and higher NAA concentrations. However, 2iP and kinetin was not significantly different at any concentration of NAA.

For shoot length of 'T63-1', F-tests showed that cytokinin type, cytokinin level, NAA, and interactions of cytokinin type and cytokinin level were highly significant at the 1% level (Table 5-3). For shoot

elongation, kinetin was most effective (2.76 cm / shoot) among 3 cytokinins and 2iP was least effective (1.37 cm / shoot). Cytokinin inhibited shoot elongation as cytokinin concentration increased (Table 5-2). The control with no cytokinin produced the longest shoots (3.18 cm / shoot), while the highest cytokinin concentration yielded the shortest ones (1.12 cm / shoot). In general, high cytokinin concentration inhibited shoot elongation of 'T63-1', and the highest cytokinin concentration (80 uM) resulted in the strongest inhibition of shoot elongation. The mean shoot length per shoot of 'T63-1' was affected by an interaction between cytokinin type and level (Fig. 5-3). BA and 2iP continued to cause shorter shoots as cytokinin concentrations increased. Kinetin produced the longest shoot length with an optimal concentration of 10 uM and then continued to produce shorter shoot length with increasing concentrations. In general, the higher cytokinin concentrations produced the shorter shoots. NAA significantly enhanced shoot elongation, but no significant differences were observed among 2, 4, and 6 uM NAA treatments (Table 5-4). NAA at 4 uM produced the longest shoots (2.16 cm / shoot), while the control with no NAA yielded the shortest ones (1.46 cm / shoot). An interaction between cytokinin type or level and NAA did not affect shoot elongation for 'T63-1' (Table 5-3).

Cytokinin type, cytokinin level, and cytokinin level * cytokinin type were all significantly different at the 1% level for explant diameter (Table 5-5). BA produced a larger explant diameter than other cytokinins (Fig. 5-4). Otherwise, kinetin yielded the smallest explant diameter. The highest cytokinin concentration resulted in the largest explant

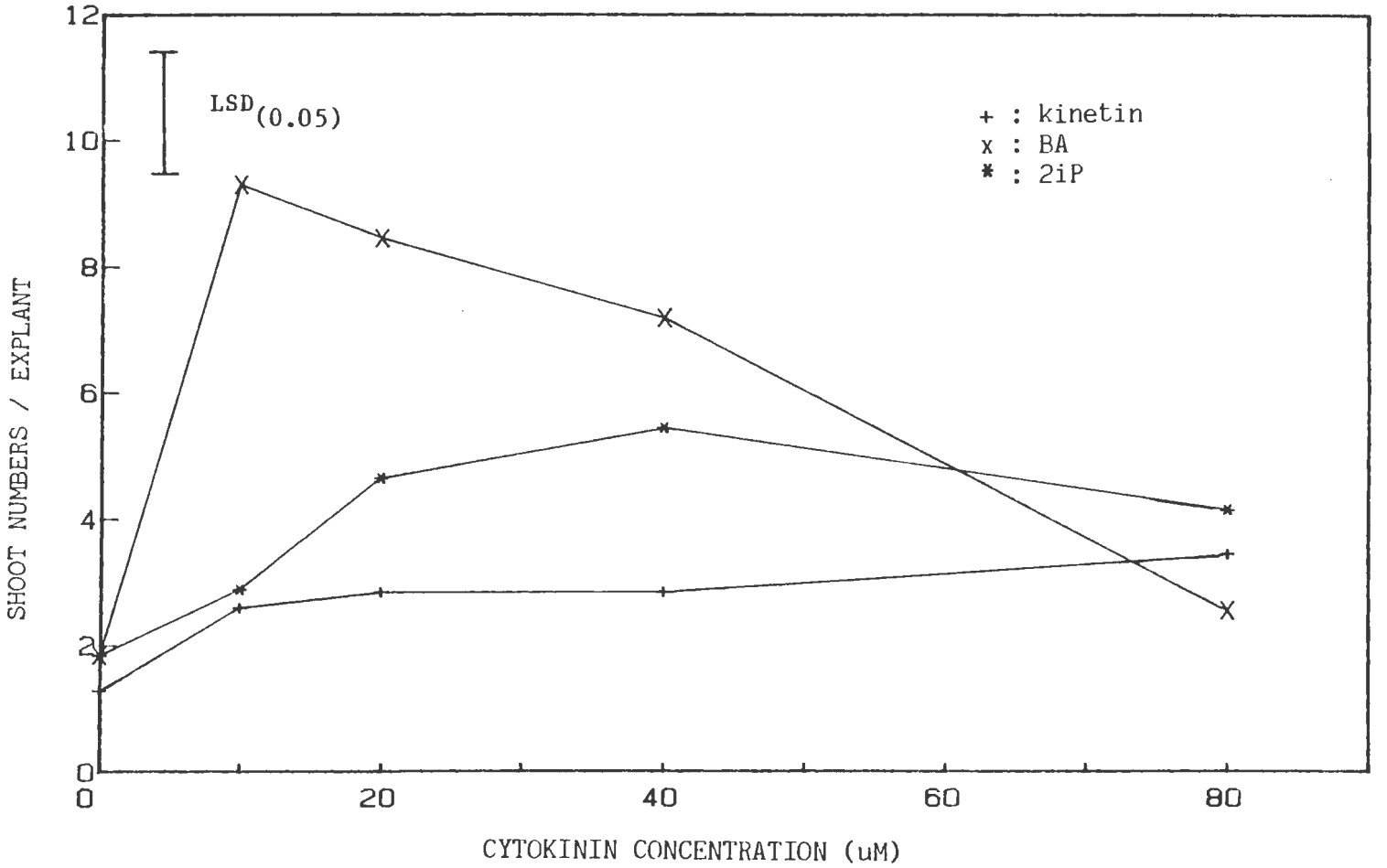

Figure 5-1. The effect of 3 cytokinins over 5 concentrations on the mean shoot number per explant of Impatiens sp. 'T63-1' after 6 weeks in culture

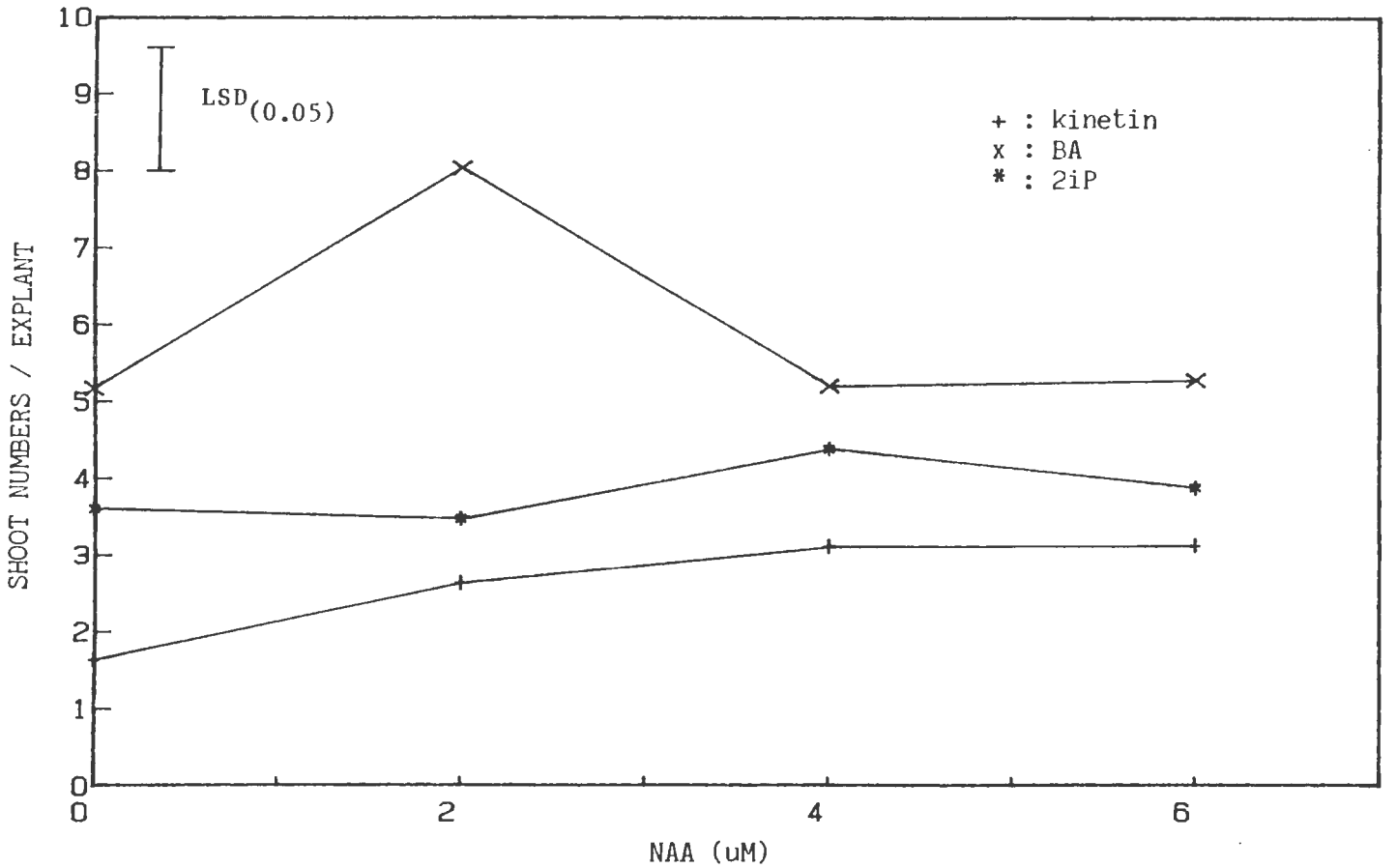

Figure 5-2. The effect of 3 cytokinins over 4 NAA concentrations on the mean shoot number per explant of Impatiens sp. 'T63-1' after 6 weeks in culture

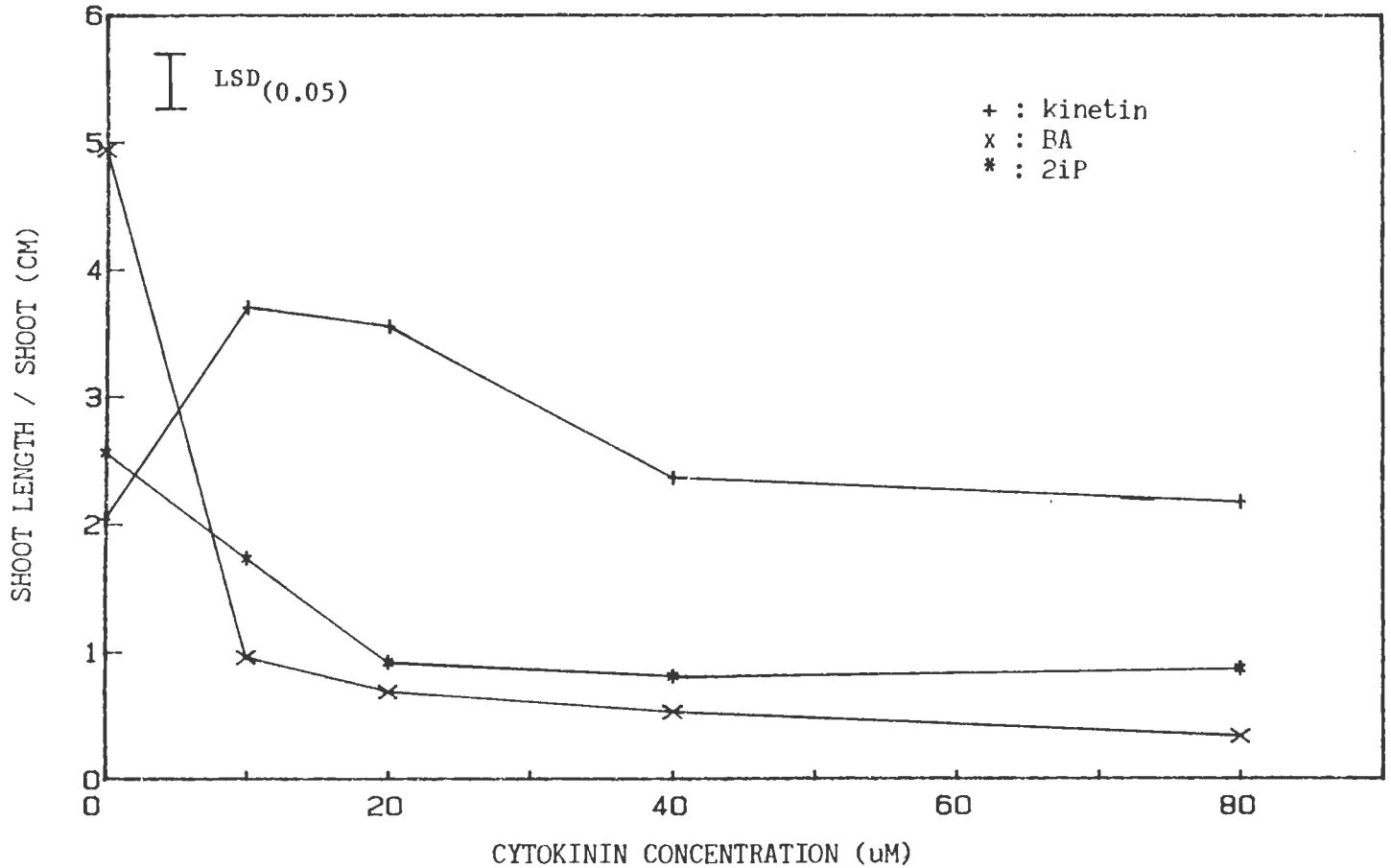

Figure 5-3. The effect of 3 cytokinins over 5 cytokinin concentrations on the mean shoot length per shoot of Impatiens sp. 'T63-1' after 6 weeks in culture

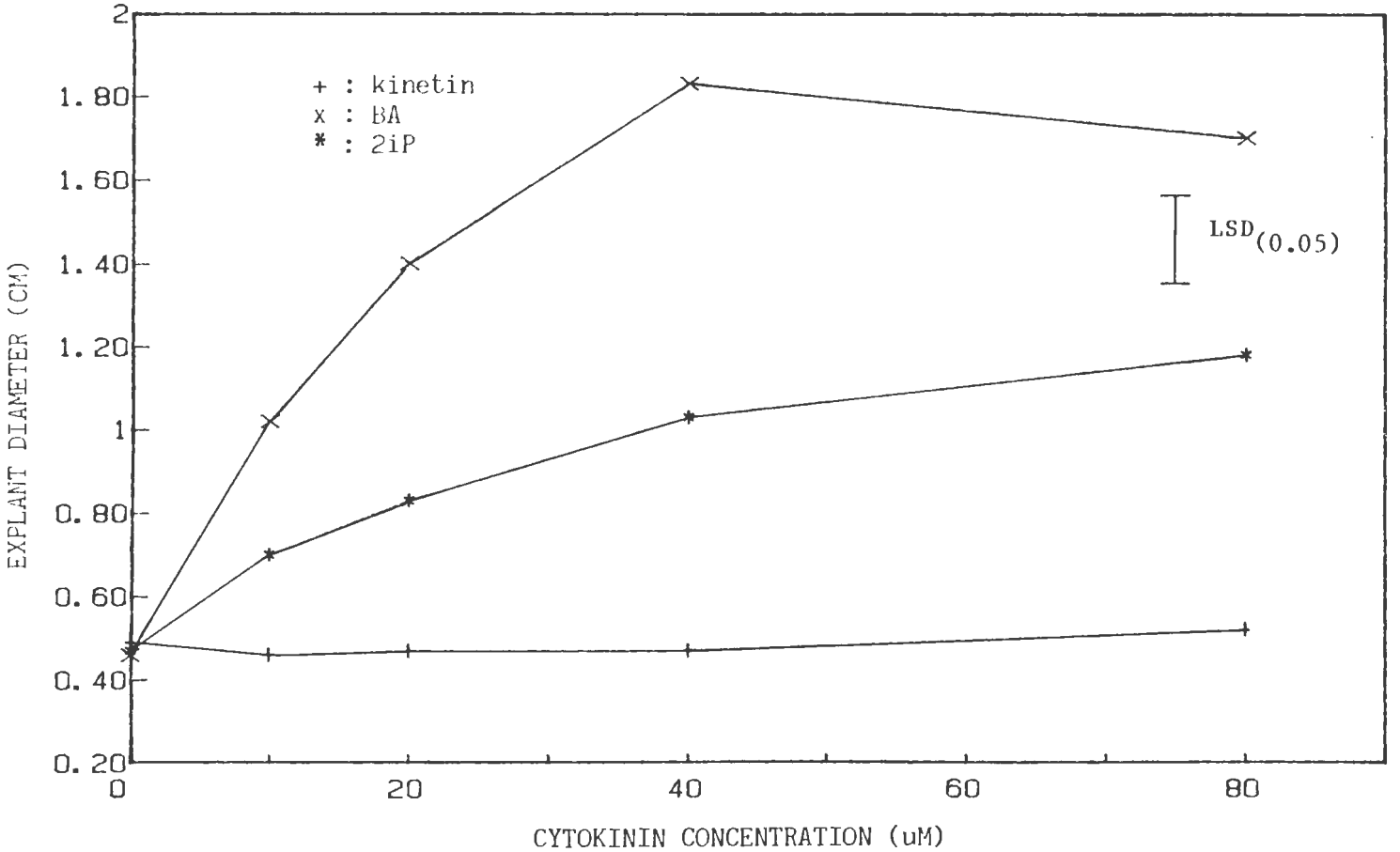

Figure 5-4. The effect of 3 cytokinins over 5 cytokinin concentrations on the explant diameter of Impatiens sp. 'T63-1' after 6 weeks in culture

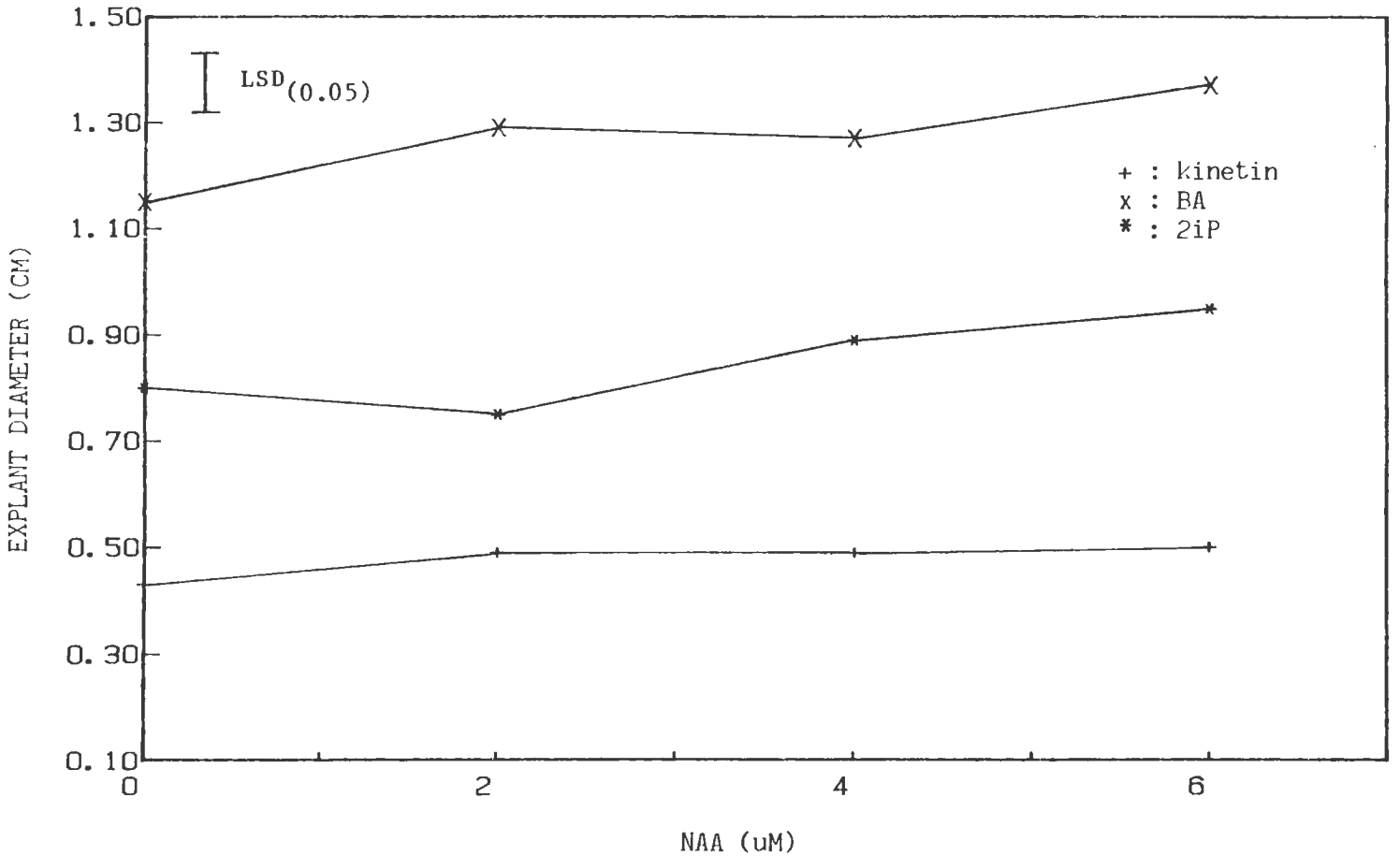

Figure 5-5. The effect of 3 cytokinins over 4 NAA concentrations on the explant diameter of Impatiens sp. 'T63-1' after 6 weeks in culture

Table 5-1. Analysis of variance for the number of shoots per shoot-tip explant of Impatiens sp. 'T63-1' after 6 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	80.60	2.21	0.0691
Cytokinin type	2	556.38	30.47	0.0001**
Cytokinin level	4	573.24	15.70	0.0001**
Cyto. type * cyto. level	8	560.45	7.67	0.0001**
NAA	3	62.92	2.30	0.0784
Cyto. type * NAA	6	142.31	2.60	0.0187*
Cyto. level * NAA	12	128.03	1.17	0.3068
Cyto.type*cyto.level*NAA	24	247.97	1.13	0.3102
Error	230	2099.93		

*p<.05.

**p<.01.

Table 5-2. Effect of 5 cytokinin concentrations on the means of shoot number, shoot length, root number, and explant diameter of Impatiens sp. 'T63-1'

Cytokinin conc.(uM)	'T63-1'			
	SN[w]	SL(cm)[x]	ED(cm)[y]	RN[z]
0	1.67d[v]	3.18a	0.47d	4.51a
10	4.97bc	2.14b	0.73c	3.34a
20	5.32a	1.72bc	0.90b	2.03b
40	5.17ab	1.24cd	1.11a	1.07bc
80	3.41c	1.12cd	1.11a	0.57c
$LSD_{0.05}$	1.08	0.47	0.12	1.23

[w]Shoot number/explant.

[x]Shoot length/explant.

[y]Explant diameter.

[z]Root number/explant.

[v]Mean comparison in column by LSD test at the 5% level.

Table 5-3. Analysis of variance for the shoot length of Impatiens sp. 'T63-1' per shoot after 6 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	8.72	1.26	0.2877
Cytokinin type	2	117.89	33.97	0.0001**
Cytokinin level	4	167.17	24.09	0.0018**
Cyto. type * cyto. level	8	232.23	16.73	0.0001**
NAA	3	22.15	4.26	0.0006**
Cyto. type * NAA	6	11.09	1.07	0.3839
Cyto. level * NAA	12	29.44	1.41	0.1601
Cyto.type*cyto.level*NAA	24	46.02	1.11	0.3386
Error	236	409.45		

**$p<.01$.

Table 5-4. Effect of 4 NAA concentrations on the means of shoot number, shoot length, root number, and explant diameter of *Impatiens* sp. 'T63-1'

NAA Conc.(uM)	'T63-1'			
	SN[W]	SL(cm)[X]	ED(cm)[Y]	RN[Z]
0	3.44b[V]	1.46b	0.79b	1.03c
2	4.72a	1.82ab	0.85ab	2.04bc
4	4.23ab	2.16a	0.89ab	3.64a
6	4.11ab	2.08a	0.95a	2.42b
$LSD_{0.05}$	0.98	0.42	0.11	1.11

[W]Shoot number/explant.

[X]Shoot length/explant.

[Y]Explant diameter.

[Z]Root number/explant.

[V]Mean comparison in column by LSD test at the 5% level.

Table 5-5. Analysis of variance for the explant diameter of Impatiens sp. 'T63-1' after 6 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	2.58	5.66	0.0002**
Cytokinin type	2	31.83	139.42	0.0001**
Cytokinin level	4	17.07	37.40	0.0001**
Cyto. type * cyto. level	8	11.82	12.95	0.0001**
NAA	3	0.69	2.02	0.1112
Cyto. type * NAA	6	0.37	0.55	0.7719
Cyto. level * NAA	12	0.88	0.64	0.8077
Cyto.type*cyto.level*NAA	24	1.53	0.56	0.9540
Error	230	26.25		

** $p<.01$.

Table 5-6. Analysis of variance for the number of roots of *Impatiens* sp. 'T63-1' per shoot-tip explant after 3 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	54.93	1.16	0.3294
Cytokinin type	2	701.47	29.62	0.0001**
Cytokinin level	4	616.05	13.01	0.0001**
Cyto. type * cyto. level	8	1115.04	11.79	0.0001**
NAA	3	260.02	7.32	0.0001**
Cyto. type * NAA	6	282.69	3.98	0.0008**
Cyto. level * NAA	12	398.44	2.80	0.0013**
Cyto.type*cyto.level*NAA	24	576.47	2.03	0.0042**
Error	230	2723.32		

** $p<.01$.

diameter (Table 5-2). Explant diameter increased with increasing cytokinin concentrations. BA produced the largest explant diameter at 40 uM after 6 weeks in culture, while kinetin did not affect explant diameter so much with increasing concentrations (Fig. 5-4). The cytokinin, 2iP, continued to produce larger explant diameter over various concentrations but still smaller diameters than those in medium with BA. NAA seemed to stimulate explant growth as concentration increased (Fig. 5-5) but was not statistically significant (Table 5-5).

An analysis of variance table for root numbers showed that cytokinin type, cytokinin level, cytokinin type * cytokinin level, NAA, cytokinin type * NAA, cytokinin level * NAA were all highly significant at the 1% level after 3 weeks in culture (Table 5-6). Table 5-2 shows that root formation was inversely proportional to cytokinin concentration. Higher cytokinin concentrations resulted in less root formation. The control with no cytokinins was most effective for causing root formation of 'T63-1'. NAA stimulated root formation, and NAA at 4 uM produced the most roots among 4 NAA concentrations tested (Table 5-4).

B. Experiment II: 'Starfire'

Shoot multiplication of Impatiens sp. 'Starfire' showed significant differences for cytokinin type, cytokinin level, and cytokinin type * cytokinin level (Table 5-7). Replication differences were also observed at the 1% level. Cytokinin types clearly affected shoot multiplication of 'Starfire'. The cytokinin 2iP produced the most shoots per explant of 'Starfire', and kinetin produced the least. Shoot multiplication was

stimulated by cytokinin concentrations from 10-40 uM (Table 5-8). Even though the highest shoot numbers were observed with a cytokinin concentration of 20 uM, there were no significant differences observed between 10 and 40 uM. Mean shoot numbers with no cytokinins were only 1.40 shoots per explant. For the combined effects of cytokinin type * cytokinin level on shoot multiplication, 2iP produced the most shoots at 40 uM and higher concentrations inhibited shoot production, while BA stimulated shoot multiplication slightly at 10 uM but steadily inhibited shoot production with increasing concentrations (Fig. 5-6). Compared to other cytokinins, kinetin concentrations had the least effect on shoot multiplication, and kinetin showed no clear trend with increasing concentrations (Fig. 5-6). There were highly significant differences for NAA and cytokinin level * NAA at the 1% level, and cytokinin types * NAA at the 5% level for shoot multiplication (Table 5-7). NAA concentrations were inversely proportional to shoot multiplication for 'Starfire' (Table 5-9). Treatment with no NAA showed the highest shoot multiplication and the highest NAA concentration resulted in the fewest shoots produced. In general, shoot multiplication was inversely affected by increasing NAA concentrations over all cytokinin levels except one combination with 2iP at 2 uM NAA (Fig. 5-7). The highest concentration of NAA resulted in the lowest shoot multiplication, regardless of cytokinin type or concentration. F-tests also showed that NAA and cytokinin concentrations interacted to significantly affect shoot multiplication (Fig. 5-8). The concentration of 2 uM NAA produced a peak in shoot production at 20 uM cytokinin, while 6 uM NAA inhibited shoot production at all cytokinin

levels.

Shoot length of 'Starfire' was significantly different for cytokinin level and cytokinin type * cytokinin level at the 1% level, based on an F-test (Table 5-10). Cytokinin type alone did not affect shoot elongation of 'Starfire'. Shoot elongation was generally suppressed as cytokinin concentration increased (Table 5-8). The highest cytokinin concentrations produced the shortest shoots (0.46 cm / shoot), while the control with no cytokinin concentration yielded the longest shoot length per shoot (1.15 cm). The mean shoot length per shoot of 'Starfire' was affected by cytokinin type and cytokinin level (Fig. 5-9). The cytokinin 2iP inhibited shoot growth at 80 uM, while kinetin had no effect on shoot elongation. BA inhibited shoot elongation and produced shorter shoots as concentrations increased. NAA, cytokinin type * NAA, and cytokinin level * NAA were significant at the 1% level (Table 5-10). The second order interaction of cytokinin type * cytokinin level * NAA was also significant for shoot elongation at the 5% level. NAA showed very similar trends to those of cytokinin concentrations (Table 5-9). The highest NAA yielded the shortest shoot length (0.51 cm / shoot), while the control with no NAA produced the longest shoots (1.22 cm / shoot). The higher that NAA concentrations were, the more that shoot elongation was inhibited. The mean shoot length per shoot of 'Starfire' was affected by 3 cytokinins over 4 levels of NAA (Fig. 5-10). Shoot elongation was inhibited by with increasing NAA concentration in the presence of kinetin or BA, but shoot elongation was not affected by increasing NAA concentration in the presence of 2iP. Shoot elongation of 'Starfire' was generally inhibited

with increasing NAA concentrations.

An analysis of variance for explant diameter of 'Starfire' per shoot-tip after 6 weeks in culture indicated that cytokinin type and cytokinin type * cytokinin level were highly significant at the 1% level (Table 5-11). Compared to other cytokinins, BA produced a slightly smaller explant diameter than kinetin or 2iP. Cytokinin concentration alone did not affect explant diameter of 'Starfire' (Table 5-8). The interaction effect of cytokinin type and cytokinin level on explant diameter indicated that BA was significantly different from kinetin and 2iP because BA produced a smaller explant diameter, while kinetin and 2iP caused a slight increase in diameter with increasing cytokinin concentration (Fig. 5-12). Explant diameter was strongly affected by NAA concentrations (Table 5-9). Cytokinin type * NAA, and cytokinin type * cytokinin level * NAA were also highly significant at the 1% level and cytokinin level * NAA was significant at the 5% level (Table 5-11). As NAA concentration increased, explant diameter also increased (Table 5-9). The highest NAA concentration produced the largest explant diameter. No matter what the cytokinin type, explant diameter increased with increasing NAA concentrations (Fig. 5-13). In general, a higher NAA concentration produced a larger explant diameter, regardless of cytokinin concentration (Fig. 5-14).

F-tests showed that root formation of 'Starfire' was affected by cytokinin type, cytokinin level, cytokinin type * cytokinin level, NAA and cytokinin level * NAA at the 1% level and cytokinin type * NAA at the 5% level after 3 weeks in culture (Table 5-12). Root formation was inhibited

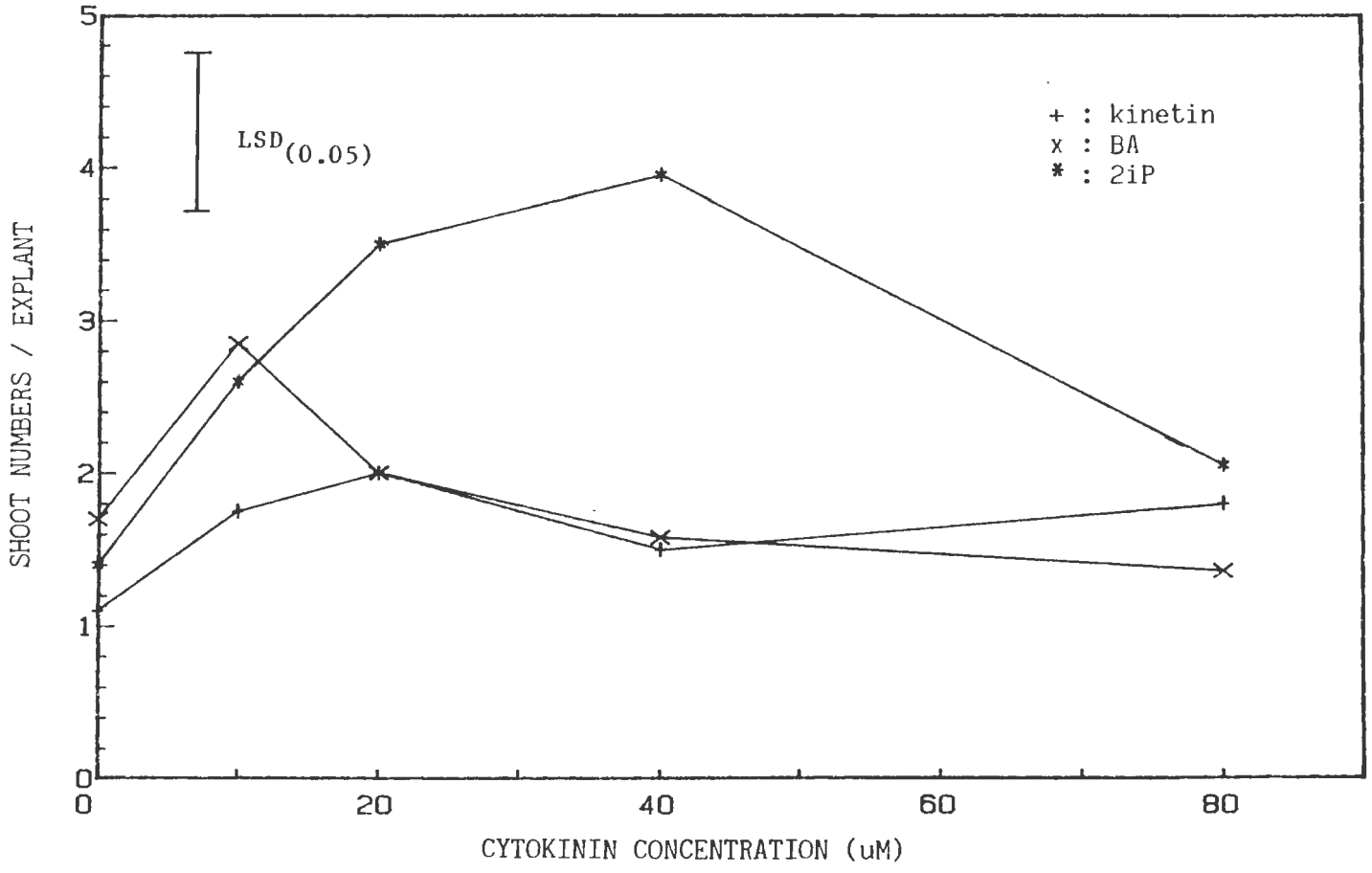

Figure 5-6. The effect of 3 cytokinins on the mean shoot number per explant of Impatiens sp. 'Starfire' after 6 weeks in culture

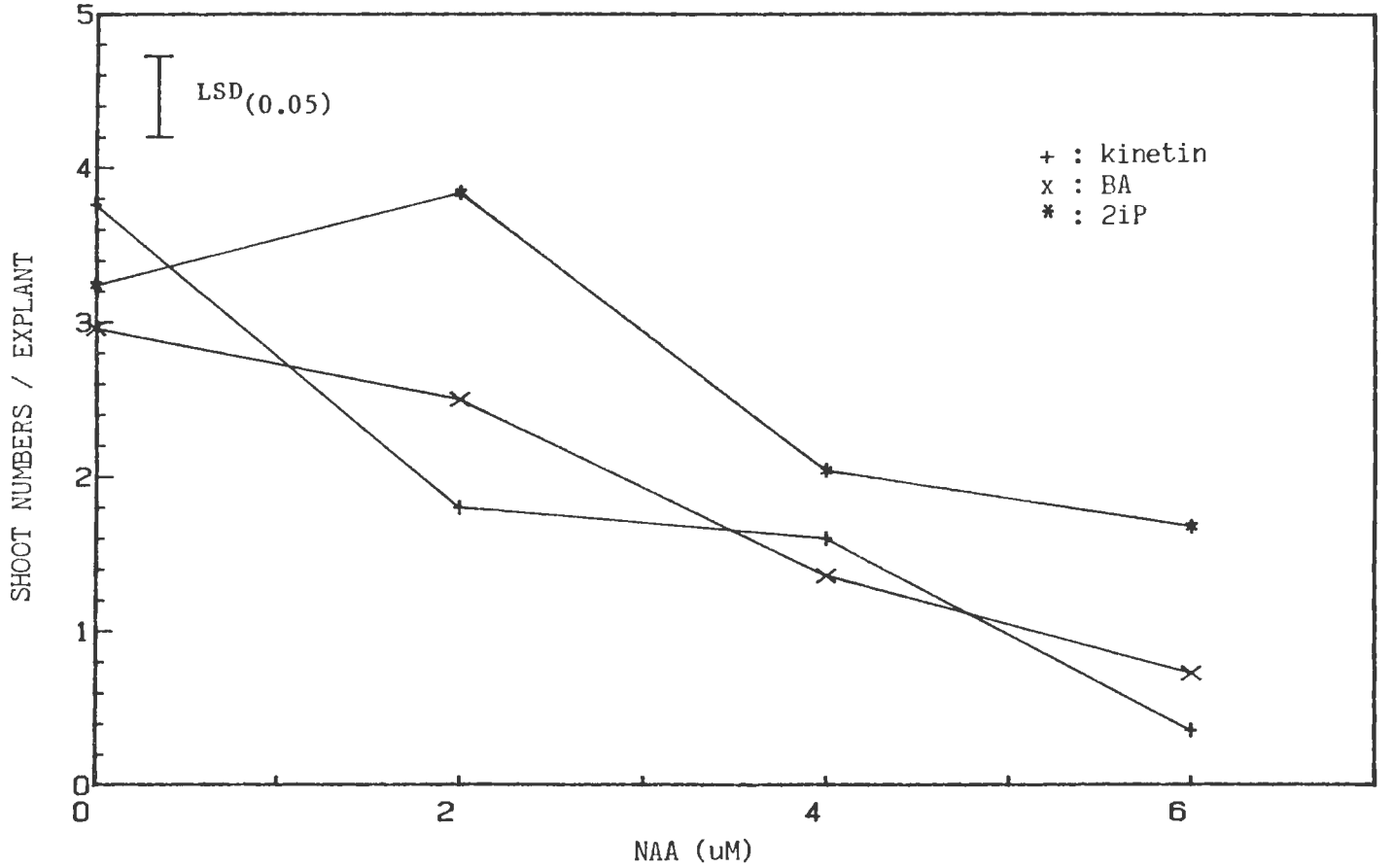

Figure 5-7. The effect of 3 cytokinins over 4 NAA concentrations on the mean shoot number per explant of Impatiens sp. 'Starfire' after 6 weeks in culture

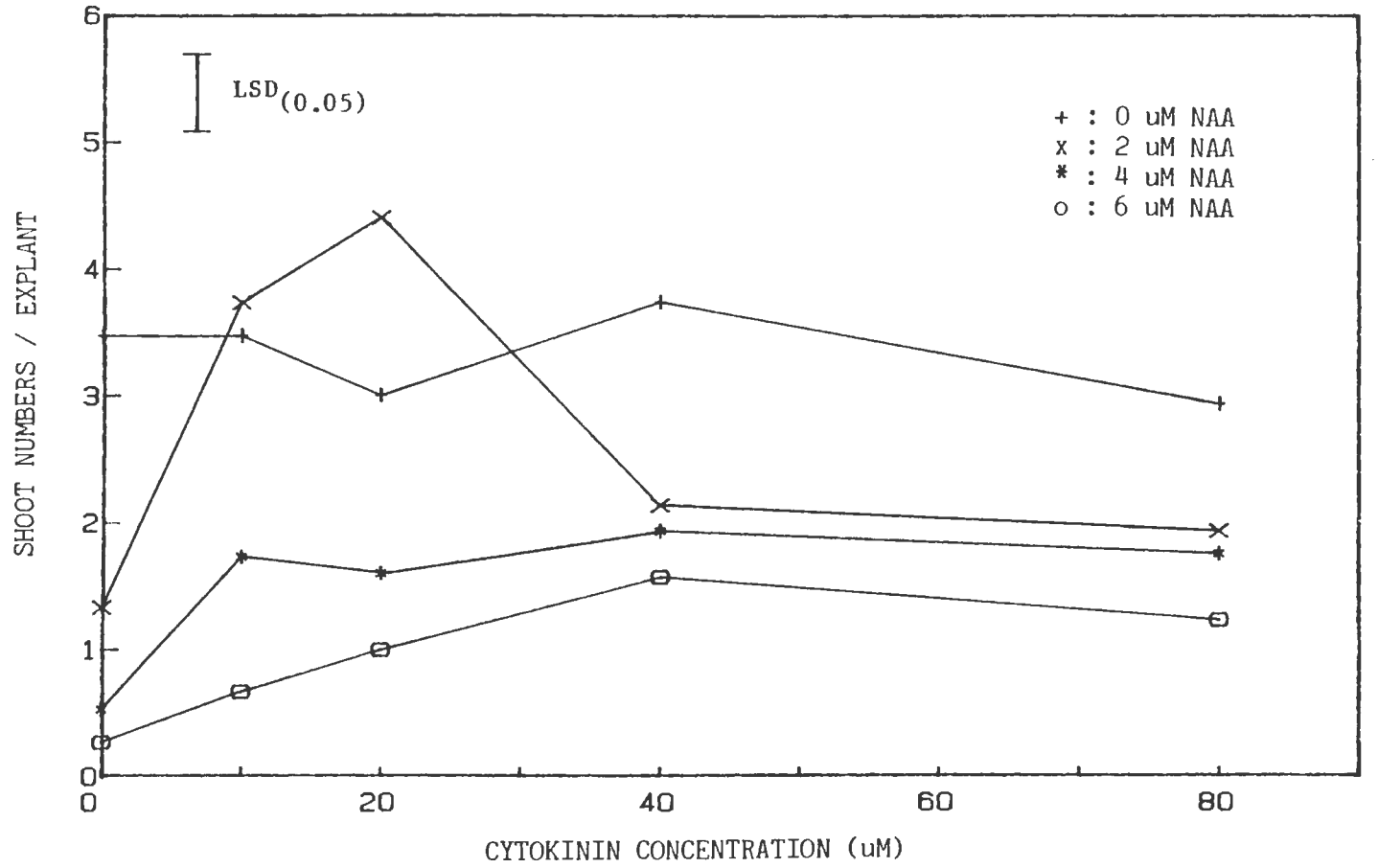

Figure 5-8. The effect of NAA over combined cytokinins and 5 concentrations on the mean shoot number per explant of Impatiens sp. 'Starfire' after 6 weeks in culture

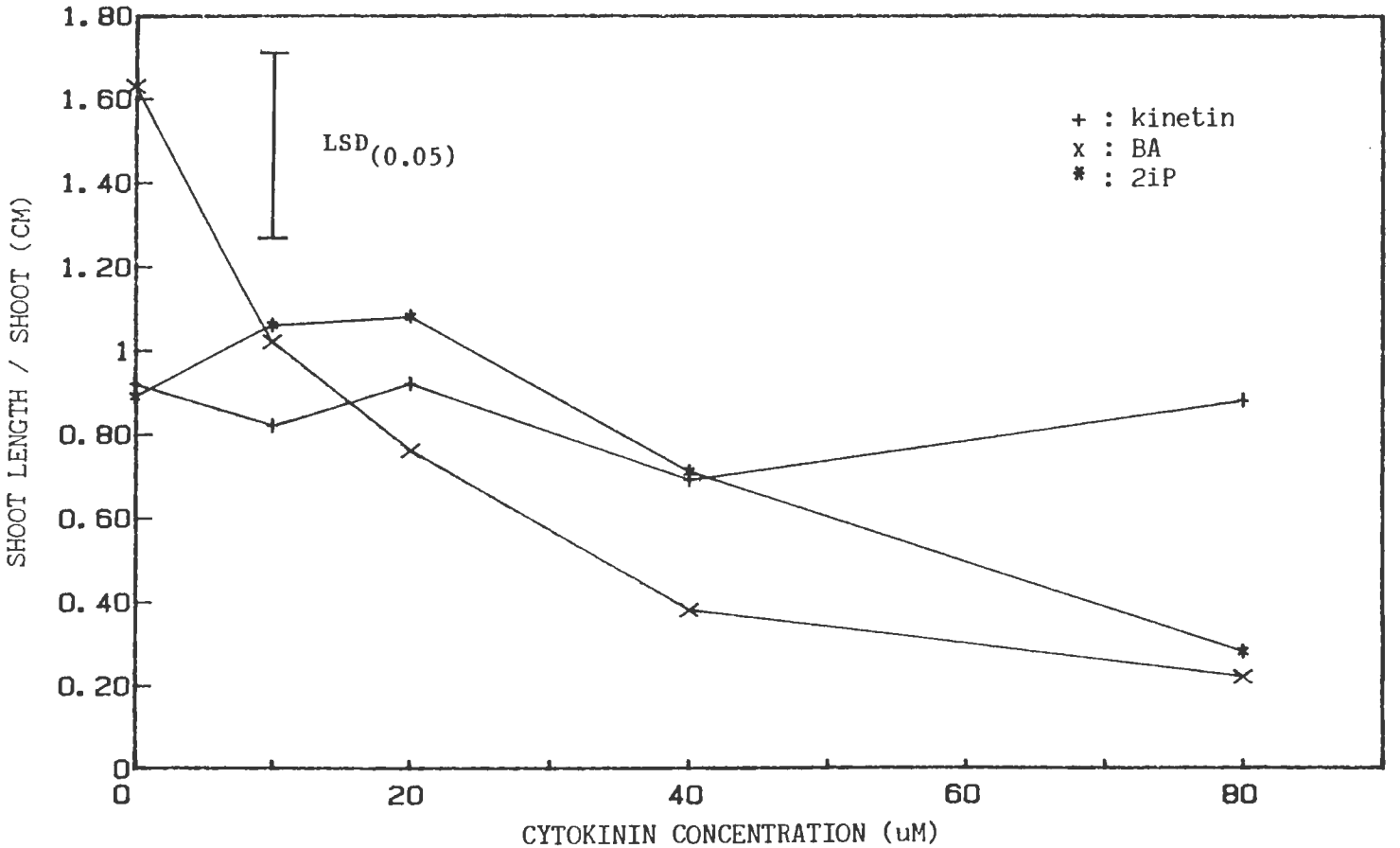

Figure 5-9. The effect of 3 cytokinins on the mean shoot length per shoot of Impatiens sp. 'Starfire' after 6 weeks in culture

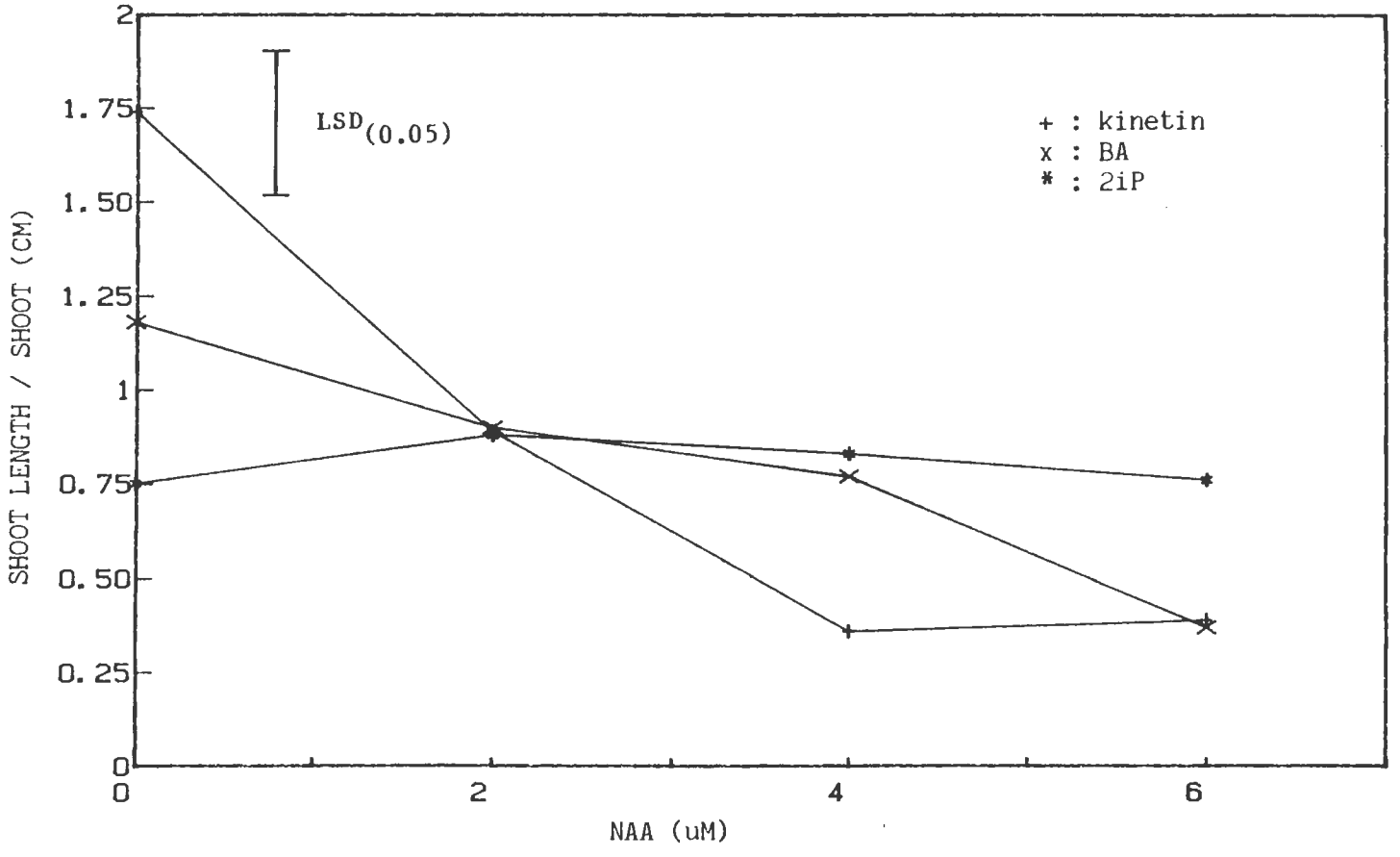

Figure 5-10. The effect of 3 cytokinins over 4 NAA concentrations on the mean shoot length per shoot of Impatiens sp. 'Starfire' after 6 weeks in culture

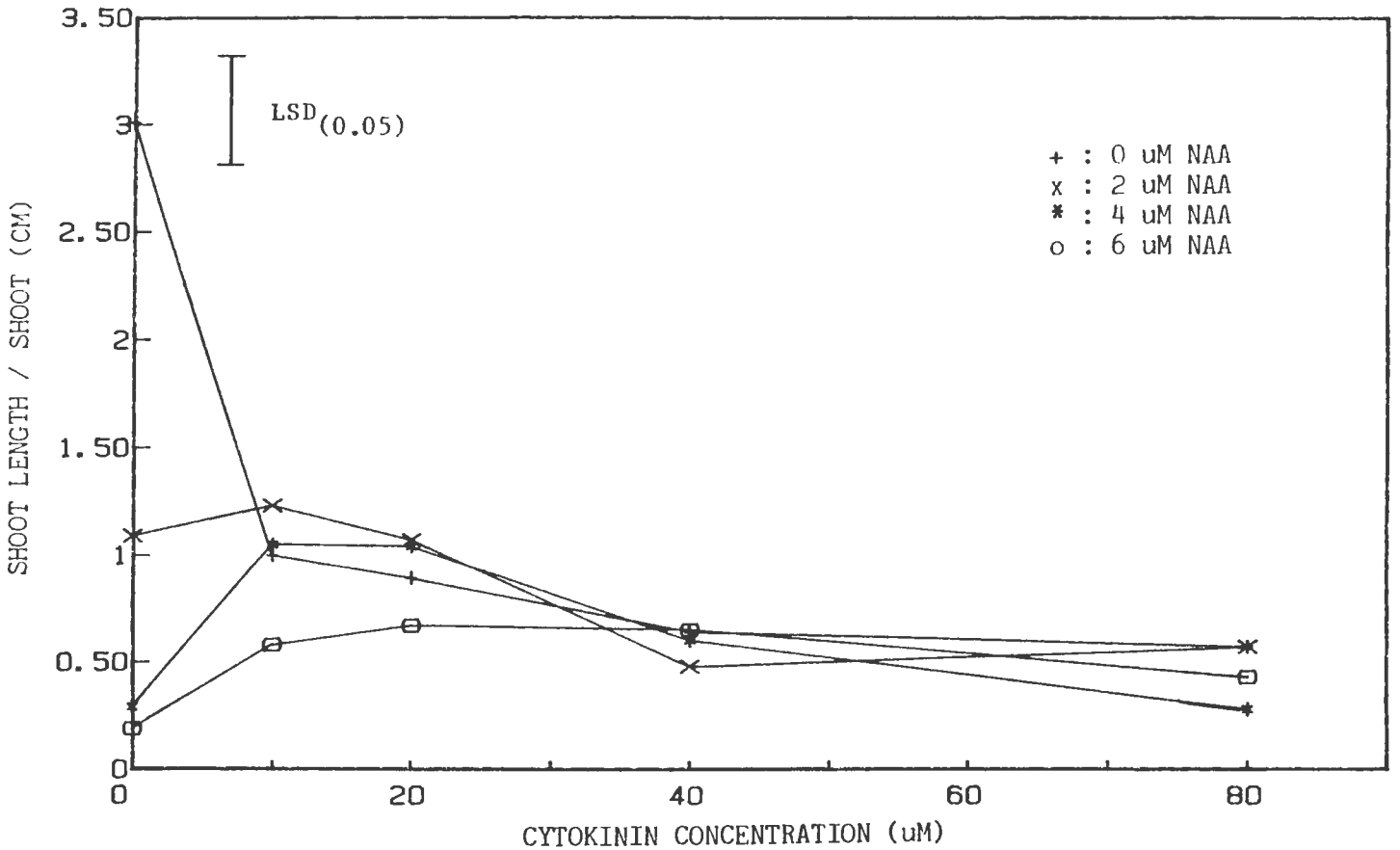

Figure 5-11. The effect of 4 NAA concentrations over combined cytokinins and 5 concentrations on the mean shoot length per shoot of Impatiens sp. 'Starfire' after 6 weeks in culture

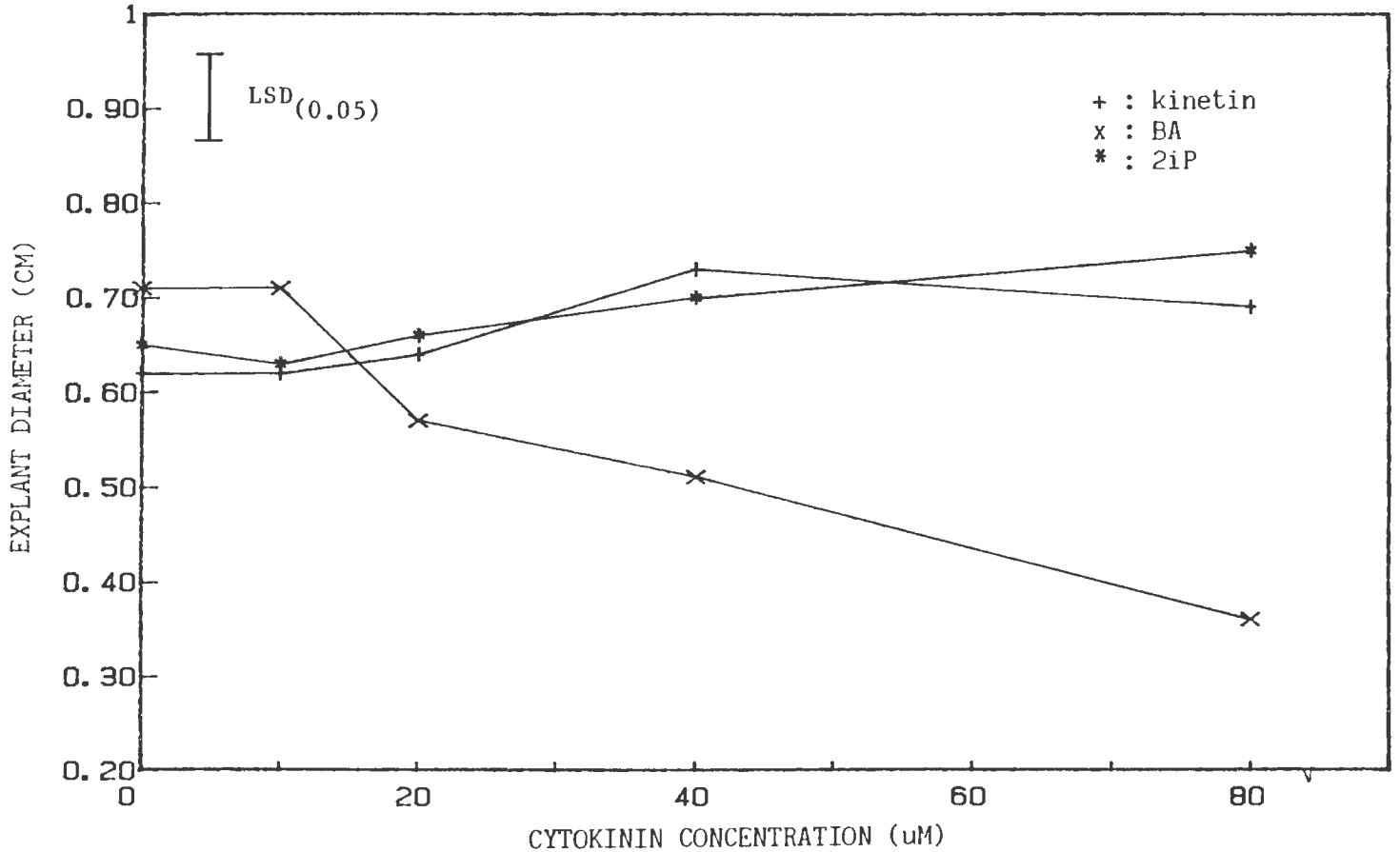

Figure 5-12. The effect of 3 cytokinins on explant diameter of Impatiens sp. 'Starfire' after 6 weeks in culture

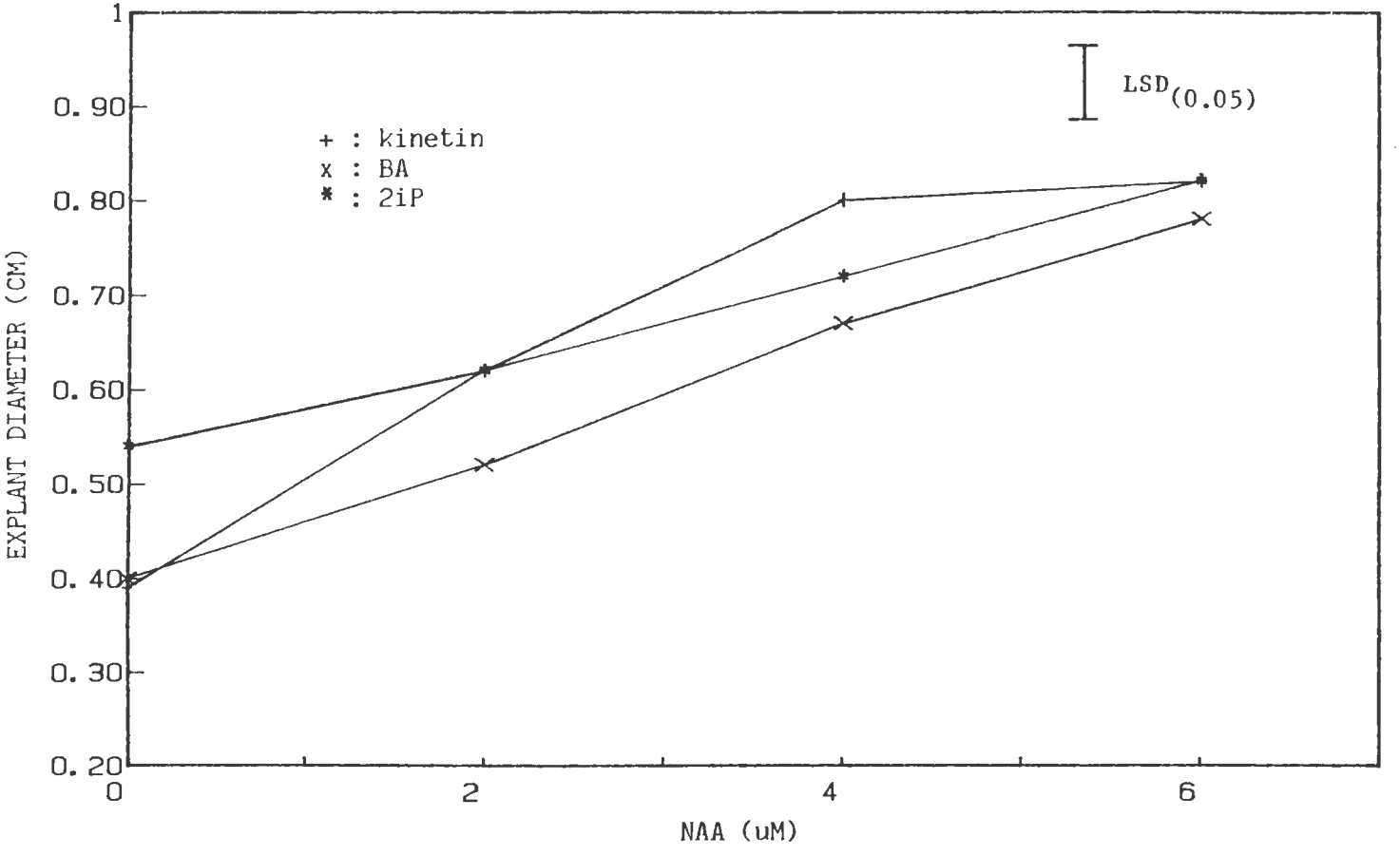

Figure 5-13. The effect of 3 cytokinins over 4 NAA concentrations on explant diameter of Impatiens sp. 'Starfire' after 6 weeks in culture

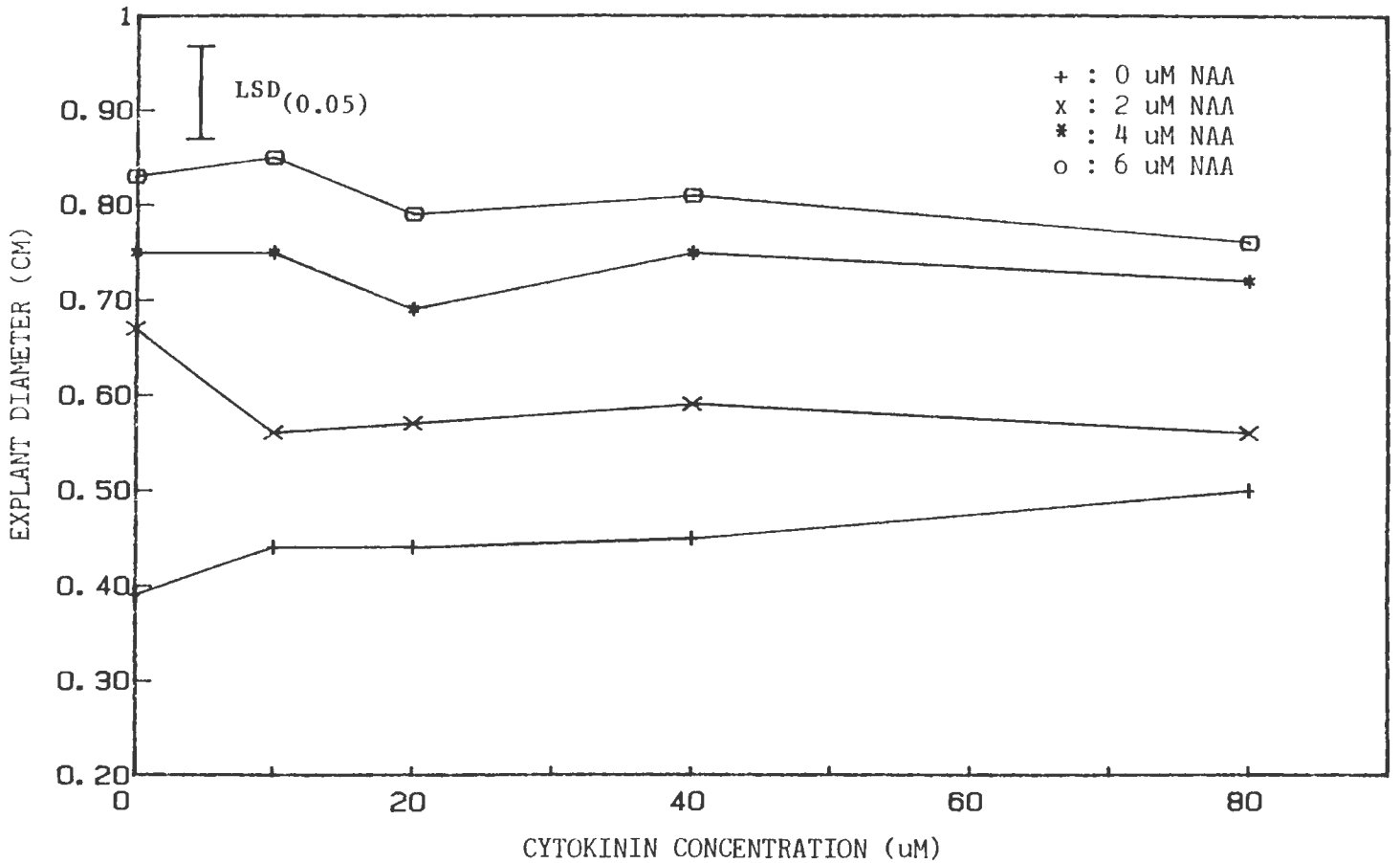

Figure 5-14. The effect of 4 NAA concentrations over combined cytokinins and 5 concentrations on explant diameter of Impatiens sp. 'Starfire' after 6 weeks in culture

Table 5-7. Analysis of variance for the number of shoots of Impatiens sp. 'Starfire' per shoot-tip explant after 6 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	64.62	5.64	0.0002**
Cytokinin type	2	60.48	10.55	0.0001**
Cytokinin level	4	53.55	4.67	0.0012**
Cyto. type * cyto. level	8	68.92	3.01	0.0032**
NAA	3	283.15	32.94	0.0001**
Cyto. type * NAA	6	47.75	2.78	0.0126*
Cyto. level * NAA	12	87.43	2.54	0.0036**
Cyto.type*cyto.level*NAA	24	52.46	0.76	0.7807
Error	229	656.15		

*p<.05.

**p<.01.

Table 5-8. Effect of 5 cytokinin concentrations on the means of shoot number, shoot length, root number, and explant diameter of Impatiens sp. 'Starfire'

Cytokinin con.(uM)	'Starfire'			
	SN[W]	SL(cm)[X]	ED(cm)[Y]	RN[Z]
0	1.40c[V]	1.15a	0.66a	12.87a
10	2.40a	0.97a	0.65a	9.83b
20	2.50a	0.92a	0.62a	8.25b
40	2.36ab	0.59b	0.65a	5.71c
80	1.78bc	0.46b	0.63a	3.90c
$LSD_{0.05}$	0.61	0.25	0.05	2.02

[W]Shoot number/explant.

[X]Shoot length/explant.

[Y]Explant diameter.

[Z]Root number/explant.

[V]Mean comparison in column by LSD test at the 5% level.

Table 5-9. Effect of 4 NAA concentrations on the means of shoot number, shoot length, root number, and explant diameter of Impatiens sp. 'Starfire'

NAA conc.(um)	'Starfire'			
	SN[w]	SL(cm)[x]	ED(cm)[y]	RN[z]
0	3.32a[v]	1.22a	0.44d	5.93b
2	2.72b	0.89b	0.59c	9.65a
4	1.33c	0.65c	0.73b	9.68a
6	0.93c	0.51c	0.81a	7.56b
$LSD_{0.05}$	0.55	0.66	0.04	1.83

[w]Shoot number/explant.

[x]Shoot length/explant.

[y]Explant diameter.

[z]Root number/explant.

[v]Mean comparison in column by LSD test at the 5% level.

Table 5-10. Analysis of variance for the shoot length of Impatiens sp. 'Starfire' per shoot after 6 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	8.73	4.61	0.0013**
Cytokinin type	2	0.12	0.13	0.8793
Cytokinin level	4	18.91	9.99	0.0001**
Cyto. type * cyto. level	8	15.29	4.04	0.0002**
NAA	3	21.87	15.40	0.0001**
Cyto. type * NAA	6	17.71	6.24	0.0001**
Cyto. level * NAA	12	60.97	10.73	0.0001**
Cyto.type*cyto.level*NAA	24	20.22	1.78	0.0165*
Error	236	111.70		

*$p<.05$.

**$p<.01$.

Table 5-11. Analysis of variance for the explant diameter of Impatiens sp. 'Starfire' after 6 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	0.20	2.75	0.0289*
Cytokinin type	2	0.47	13.20	0.0001**
Cytokinin level	4	0.07	1.00	0.4104
Cyto. type * cyto. level	8	1.49	10.46	0.0001**
NAA	3	5.63	105.22	0.0001**
Cyto. type * NAA	6	0.38	3.58	0.0021**
Cyto. level * NAA	12	0.38	1.80	0.0495*
Cyto.type*cyto.level*NAA	24	1.10	2.57	0.0002**
Error	229	4.08		

*p<.05.

**p<.01.

Table 5-12. Analysis of variance for the number of roots of Impatiens sp. 'Starfire' per shoot-tip explant, after 3 weeks in culture

Source	DF	SS	F	PR>F
Replication	4	117.82	0.92	0.4511
Cytokinin type	2	341.56	5.35	0.0053**
Cytokinin level	4	2853.28	22.36	0.0001**
Cyto. level * cyto. type	8	1772.70	6.95	0.0001**
NAA	3	683.75	7.14	0.0001**
Cyto. type * NAA	6	461.70	2.41	0.0279*
Cyto. level * NAA	12	1761.95	4.60	0.0001**
Cyto.level*cyto.type*NAA	24	1088.59	1.42	0.0978
Error	230	7337.98		

*p<.05.

**p<.01.

by cytokinin (Table 5-8). As cytokinin concentration increased, the number of roots formed became significantly lower. In general, the presence of NAA strongly stimulated root formation (Table 5-9). However, at 6 uM NAA, root formation was less than at 2 or 4 uM NAA.

C. Experiment III: 'T63-1' and 'Starfire'

Shoot multiplication of Impatiens spp. 'T63-1' and 'Starfire' showed highly significant differences for cytokinin type at the 1% level (Table 5-13). Kinetin produced fewer shoots (3.2 shoots per explant) than 2iP (4.7 shoots per explant). Kinetin was less effective at every concentration for both genotypes than was 2iP (Fig 5-15). Shoot multiplication was also affected by cytokinin level and cytokinin type * level at the 5% level (Table 5-13). The highest cytokinin concentration, 60 uM, produced the most shoots (4.8 shoots per explant), while 0 uM cytokinin yielded the fewest shoots (2.8 shoots per explant). F-tests also showed that shoot multiplication was significantly affected by genotype (Table 5-13). 'Starfire' produced 4.4 shoots per explant, whereas 'T63-1' yielded 3.5 shoots per explant over all cytokinin concentrations. There were no significant interactions between genotype and cytokinins (Table 5-13).

F-tests showed that 3 main effects and an interaction were all highly significant at the 1% level for shoot length per shoot of 'T63-1' and 'Starfire' (Table 5-14). Kinetin stimulated shoot elongation more than 2iP, with kinetin shoot length averaging 2.3 cm and 2iP yielding a mean shoot length of 1.3 cm. In general, shoot elongation was inhibited by 20

uM 2iP or higher cytokinin concentrations, while kinetin had little effect on shoot elongation, regardless of cytokinin type and genotype (Fig. 5-16). Shoot elongation was also dependent upon genotype (Table 5-14). 'T63-1' generally produced shoots that averaged 2.47 cm in length, while 'Starfire' yielded only 1.07 cm long shoots, on average, and 'Starfire' shoots were shorter than 'T63-1' shoots for every level of cytokinin tested. The interaction between cytokinin type and cytokinin level indicated that kinetin inhibited shoot elongation slightly as concentrations increased, while 2iP strongly inhibited shoot elongation at 20 uM and higher concentrations (Table 5-14 and Fig. 5-16).

Explant diameter was strongly affected by cytokinin type, cytokinin level, and cytokinin type * cytokinin level at the 1% level (Table 5-15). The cytokinin 2iP produced a larger explant diameter (0.53 cm) than kinetin (0.45 cm). Explant diameter increased steadily as 2iP concentration increased, while kinetin did not affect the explant diameter as concentration increased (Fig. 5-17). In general, 2iP promoted explant-diameter growth more than kinetin, regardless of genotype (Fig. 5-17). The explant diameter was also affected by the second-order interaction, cytokinin type * cytokinin level * genotype, at the 1% level (Table 5-15). The interaction between 2iP + 'T63-1' over 4 cytokinin concentrations produced the largest explant diameter, while kinetin + 'T63-1' did not affect the explant diameter (Fig. 5-17).

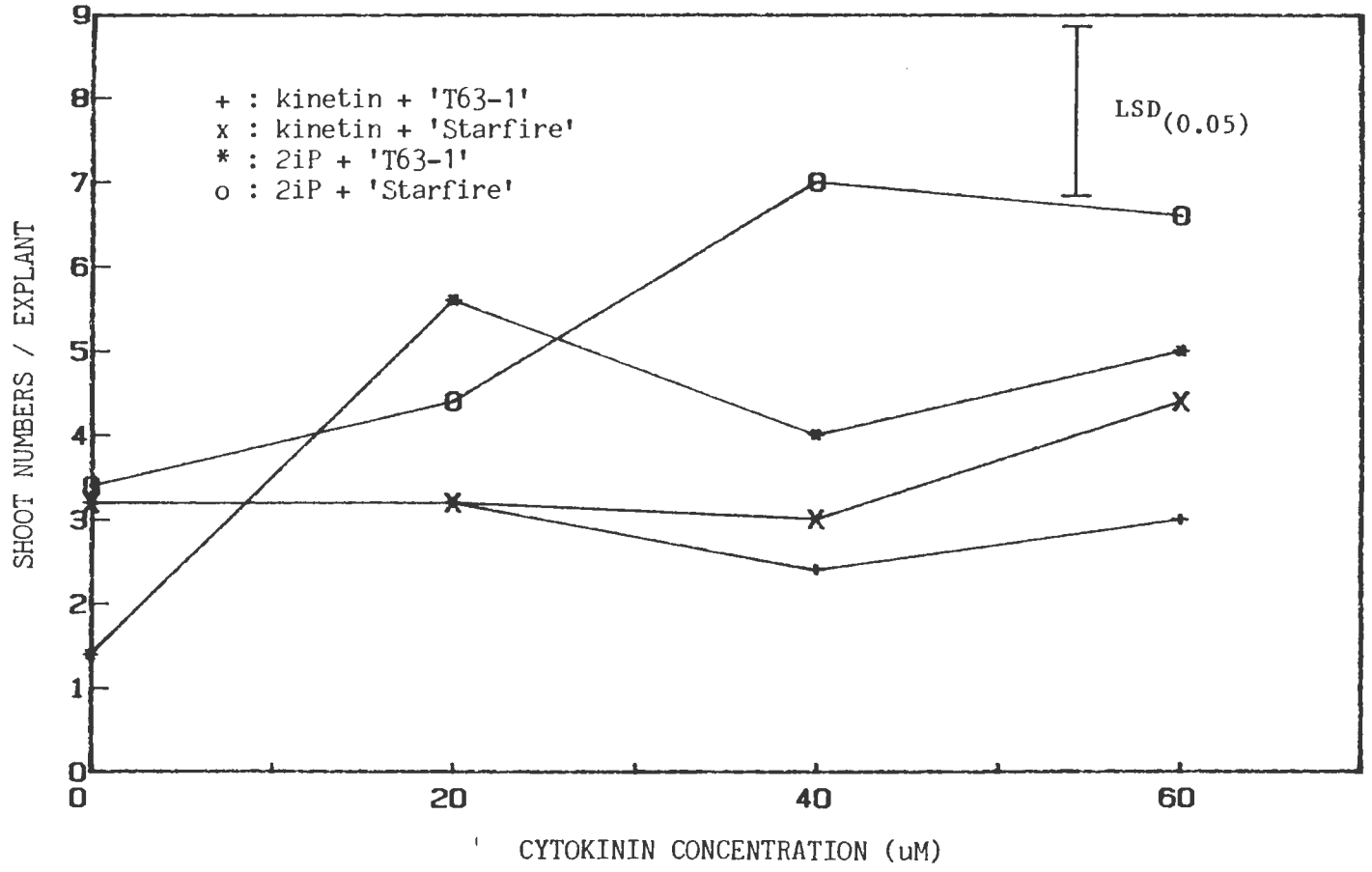

Figure 5-15. The effects of Impatiens sp. genotype and cytokinin type and level on the mean shoot number per explant after 6 weeks in culture

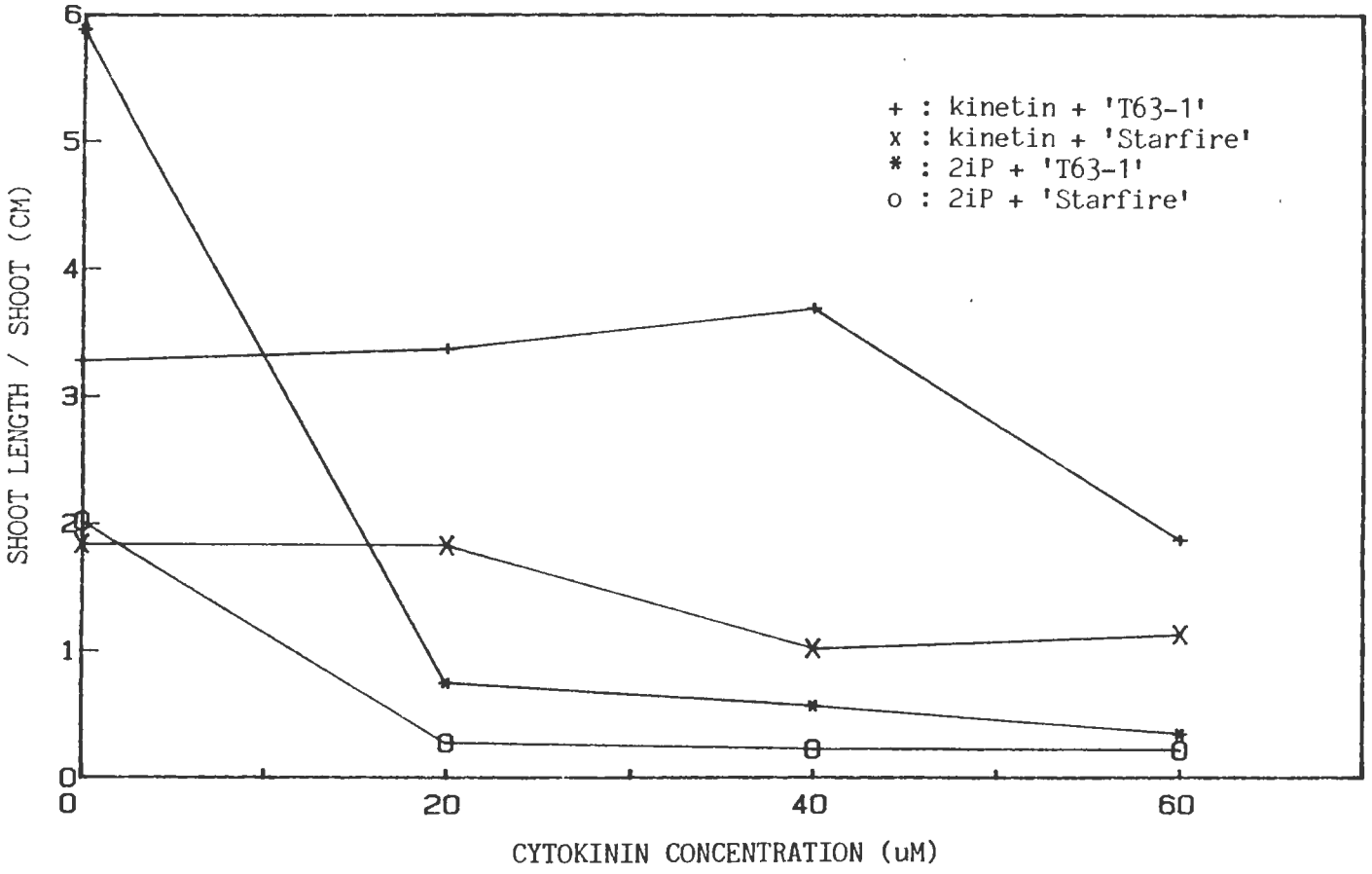

Figure 5-16. The effects of Impatiens sp. genotype and cytokinin type and level on the mean shoot length per shoot after 6 weeks in culture

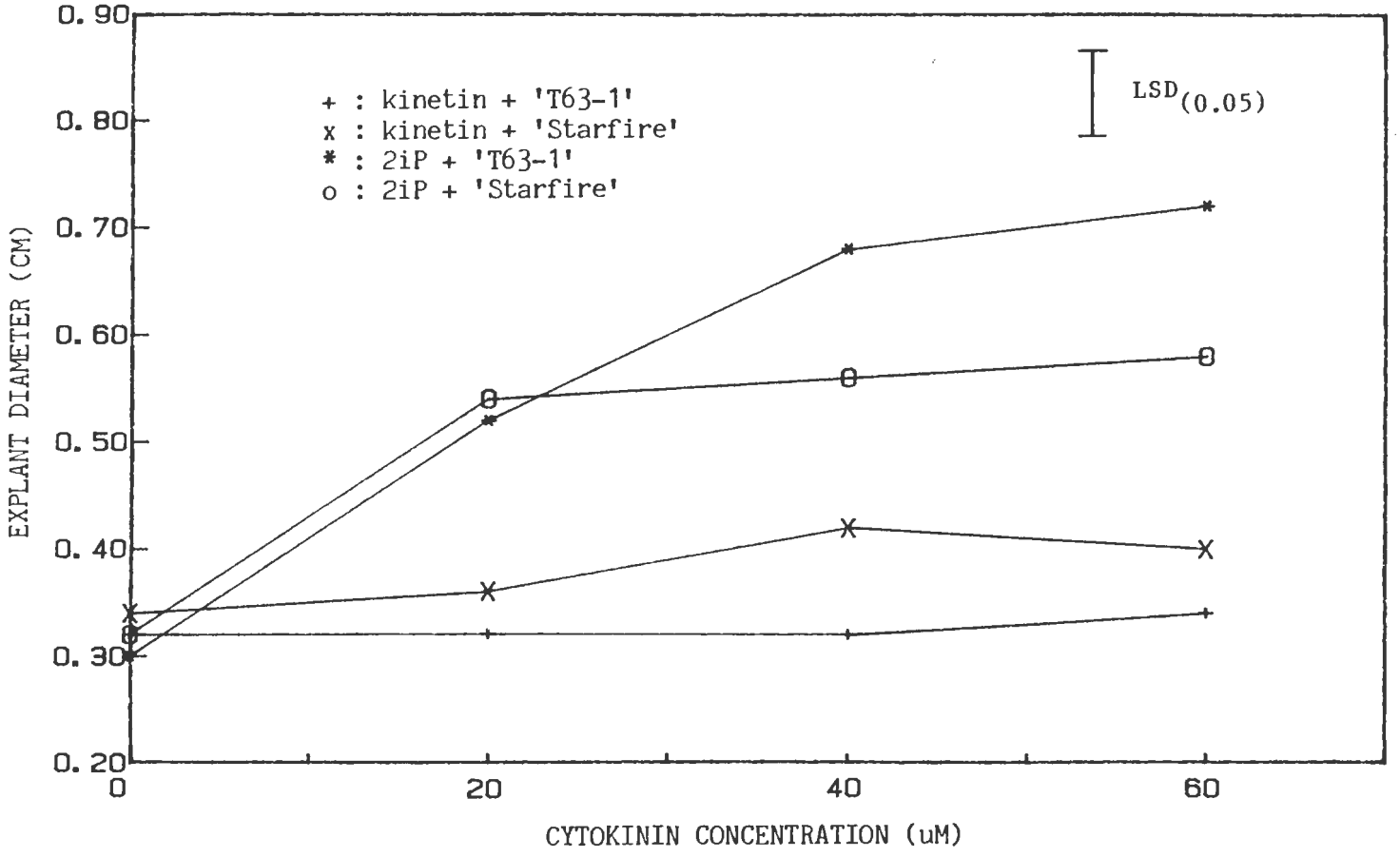

Figure 5-17. The effects of Impatiens sp. genotype and cytokinin type and level on explant diameter after 6 weeks in culture

Table 5-13. Analysis of variance for the number of shoots per explant of *Impatiens* spp. 'T63-1' and 'Starfire' after 6 weeks in culture

Source	DF	SS	F	PR>F
Cytokinin type	1	43.51	11.72	0.0011**
Cytokinin level	3	40.14	3.60	0.0180*
Cytok.type * cytok.level	3	37.14	3.33	0.0248*
Genotype	1	17.11	4.61	0.0356*
Genotype * cytok.type	1	3.61	0.97	0.3276
Genotype * cytok.level	3	17.14	1.54	0.2130
Genotype*cytok.type*level	3	10.44	0.94	0.4280

*$p<.05$.

**$p<.01$.

Table 5-14. Analysis of variance for the shoot length per shoot of *Impatiens* spp. 'T63-1' and 'Starfire' after 6 weeks in culture

Source	DF	SS	F	PR>F
Cytokinin type	1	18.58	9.56	0.0029**
Cytokinin level	3	63.46	10.89	0.0001**
Cytok.type * cytok.level	3	39.32	6.75	0.0005**
Genotype	1	39.08	20.12	0.0001**
Genotype * cytok.type	1	0.81	0.42	0.5205
Gynotype * cytok.level	3	13.32	2.29	0.0872
Genotype*cytok.type*level	3	15.24	2.61	0.0587

** $p<.01$.

Table 5-15. Analysis of variance for explant diameter of Impatiens spp. 'T63-1' and 'Starfire' after 6 weeks in culture

Source	DF	SS	F	PR>F
Cytokinin type	1	0.61	155.56	0.0001**
Cytokinin level	3	0.45	37.84	0.0001**
Cytok.type * cytok.level	3	0.28	23.24	0.0001**
Genotype	1	0.00	0.00	1.0000
Genotype * cytok.type	1	0.06	15.37	0.0002**
Genotype * cytok.level	3	0.02	1.27	0.2922
Gneotype*cytok.type*level	3	0.05	4.28	0.0082**

** $p<.01$.

VI. DISCUSSION

The results indicate that Indonesian Impatiens spp. can be rapidly propagated in vitro by an exogenous hormonal regulation. Such rapid clonal propagation using shoot-tips or meristems has been previously reported for other herbaceous ornamental plants such as carnations (Earle and Langhans 1975 and Dabiski et al. 1979), Chrysanthemum spp. (Grewal and Sharma 1978), Pelargonium spp. (Pillai and Hildebrandt 1968b), Petunia sp. (Sharma and Mitra 1976), and Veronica sp. (Stapfer et al. 1985).

Shoot multiplication of Indonesian Impatiens spp. in vitro was significantly affected by cytokinins and genotype. The requirement of cytokinin type for shoot multiplication is genotype-dependent, regardless of cytokinin level, based on experiments I, II, and III. Further, the effect of cytokinin type was consistent over all experiments. BA was most effective for shoot multiplication of 'T63-1', while 2iP was most effective with 'Starfire'. Stapfer et al. (1985) reported that BA among 3 cytokinins tested (BA, kinetin, and 2iP) was the most effective stimulator for multiple shoot production from shoot-tip culture of Veronica sp. Similar results that the cytokinin requirement was genotype-dependent were reported for Rhododendron (Fordham and Stimart 1982) and Rosaceae (Norton and Boe 1982). Kinetin did not affect shoot multiplication of 'T63-1' and 'Starfire' as much as other cytokinins. However, Stephens et al. (1985) reported that kinetin stimulated multiple shoot production in several interspecific hybrids of Impatiens spp. although very high concentrations were necessary. Apparently, both 2iP and BA were more effective than kinetin, regardless of genotype. Perhaps uptake of kinetin is less

efficient than 2iP or BA. Another possibility is that cytokinins are preferentially broken down, depending on the cytokinin oxidase operating in a given species or family (Norton and Norton 1985). An optimal cytokinin level varies, depending on genotype, but based on experiments I, II, and III, cytokinin levels between 10 and 40 uM seem to be the optimal range since a higher level has a tendency to inhibit shoot multiplication of both genotypes. BA produced the most multiple shoots at 10 uM for 'T63-1', and 2iP yielded the most multiple shoots at 40 uM for 'Starfire'. For shoot multiplication of 'Starfire', it is more realistic to assume that an optimal level is in between 40 and 60 uM because there was no significant difference between 40 and 60 uM of 2iP. The highest cytokinin level (80 uM) apparently inhibited multiple shoot production. Stapfer *et al.* (1985) obtained similar results from shoot-tip culture of *Veronica*. They found that the greatest number of shoots was produced in media containing 8 to 16 uM BA, whereas a medium containing 64 uM 2iP produced the greatest shoot number, and 2iP had only a slight stimulatory effect on shoot number at 16 uM. In shoot-tip culture of *Hosta decorata* 'Thomas Hogg', BA and 2iP were most effective at 5 mg/l and 10 mg/l for axillary and adventitious shoot multiplication, respectively (Papachatzi *et al.* 1981).

In experiments I and II, NAA inhibited shoot multiplication at all levels tested. Because of this inhibition, NAA was deleted from the media that were used for experiment III. Even when NAA is required for multiple shoot production, very low levels seem to be required. Cytokinin plus a low level of NAA (0.01 uM) produced the greatest number of shoots (Stapfer

et al. 1985), and Chaney et al. (1985) reported that there were significant differences among genotypes for shoot production with 2.0 mg/l kinetin plus 0.2 mg/l NAA from shoot-tip culture of Chrysanthemum morifolium after 8 weeks incubation. Optimal multiple shoot production was also obtained from terminal and lateral buds excised from stems of Exacum affine on a medium containing 0.01 mg/l NAA and 2.0 mg/l kinetin (Torres and Natarella 1984), whereas some researchers reported that NAA was inhibitory or not effective (Stephens et al. 1985, Bilkey and McCown 1979, Kusey et al. 1980, and Heuser 1983).

Minimal shoot length is very critical for survival of rooted shoots transferred to greenhouse conditions in most ornamental and other plants. Stephens et al. (1985) reported that 2-cm-long rooted shoots always survived, and survival of shoots smaller than 2 cm was inversely related to the shoot length in Impatiens spp. Shoot growth of Impatiens spp. showed significant differences for cytokinin type, cytokinin level, and an interaction between cytokinin type and level. The effects of cytokinin type on shoot elongation were also dependent upon genotype. For 'T63-1' in experiment I, kinetin produced the longest shoots, while in experiment II, 2iP and kinetin did not produce significantly different results for shoot length of 'Starfire', except at the highest concentration (Fig. 5-9). BA was clearly the most inhibitory to shoot elongation, so only kinetin and 2iP were compared in experiment III, where the results of experiments I and II were generally confirmed. In all 3 experiments, the highest cytokinin level inhibited shoot elongation and produced the shortest shoots, regardless of genotype. In the literature, the effects

of cytokinin on shoot elongation vary, depending on plant species and genotype tested. Olesen and Fonnesbech (1975) reported that there was an increase in shoot length with increasing cytokinin levels in shoot-tip culture of Phlox plants, while Kusey et al. (1980) reported that a level of BA or kinetin higher than 1 ppm was inhibitory for shoot elongation in in vitro propagation of Gypsophila paniculata L., and Schnabelrauch and Sink (1979) also reported that BA caused very slow shoot elongation in Phlox spp. Stapfer et al. (1985) found that BA was effective at producing shoots in the 5 mm long and longer category, whereas Samartin et al. (1984) reported that longer shoots were observed in absence of BA than in presence of BA. Shoot elongation is very closely related to the shoot number. In general, shoot elongation is inversely proportional to multiple shoot production, based on our experiments. Many researchers have found this inverse relationship between shoot elongation and shoot number in herbaceous ornamental plants and have found that it is important to strike a compromise between shoot production and shoot length (Kunisaki 1975, Earle and Langhans 1975, Lineberger and Wanstreet 1983, and Heuser 1983).

Like cytokinin concentration, shoot elongation was inhibited at high NAA concentrations. Similar results have been reported (Dabiski et al. 1979 and Lineberger and Wanstreet 1983). Other auxins such as IAA inhibited shoot elongation at a higher level than 1 uM, but stimulated shoot elongation at a lower level than 1 uM (Grewal and Sharma 1978). High auxin concentrations apparently inhibit shoot elongation in many herbaceous ornamentals growing in vitro.

The final explant size when data were taken included some callus produced within the explant. Callus growth is often genetically unstable, and shoots arising from callus have been shown to be genetically different from the original explant (Skirvin and Janick 1975). Thus, the best conditions are those that cause explants to produce the largest number of shoots with the least callus. Explant diameter in all experiments was regulated by cytokinin type, cytokinin level, an interaction between cytokinin type and level, NAA, and an interaction between NAA and cytokinin type or level. The effect of cytokinin-type on explant diameter is also genotype-dependent. BA produced the largest explant diameter for 'T63-1', while 2iP yielded the largest diameter for 'Starfire'. Kinetin did not affect explant diameter regardless of genotype. BA is most commonly used for callus production in many herbaceous ornamental plants (Sharma and Mitra 1976, Brandao and Salema 1977, and Bilkey and McCown 1979) and Kusey *et al.* (1980) reported that BA was superior to kinetin for callus production in *Gypsophila paniculata* L., whereas Dabiski *et al.* (1979) observed some callus forming on the shoots grown on 13.5 uM kinetin combined with 0.5 uM NAA. Cytokinin level has increased explant diameter with increasing concentration in our experiments. Stephens (1985) reported that increasing cytokinin level did not stimulate callus production in seedling explants of *Impatiens* spp. However, he used different explant sources and different *Impatiens* species.

NAA is very important and a well-known growth regulator for callus production. In most species, NAA stimulates callus production, as with results of our experiments I and II. NAA increased the explant diameter

significantly with increasing concentration. NAA combined with a cytokinin is more often used for callus production rather than NAA by itself. Many researchers have found NAA and cytokinin effects on callus production for several herbaceous ornamental plants (Dabiski et al. 1979, Sharma and Mitra 1976, Brandao and Salema 1977, Bilkey and McCown 1979, Banta and Torres 1984, and Torres and Natarella 1984). Explant diameter seems to affect shoot multiplication and shoot elongation. Larger explants produce more shoots, but the increase in diameter causes less growth in shoot length for 'T63-1' and 'Starfire'. A higher NAA concentration resulted in a larger diameter and produced more shoots, but resulted in shorter shoot length. In many cases, a low NAA concentration is preferred for in vitro propagation, because genetic stability of the clone is better maintained, and increased shoot multiplication may occur. Brandao and Salema (1977) reported that shoot multiplication was favored by auxin/cytokinin ratios lower than 1:100, within the concentrations tested. Torres and Natarella (1984) also reported that increased shoot production was observed at low NAA concentration. Kusey et al. (1980) and Olesen and Fonnesbech (1975) reported that a high NAA concentration stimulated callus growth but inhibited shoot elongation. There are two opposite reports for the effect of NAA on callus versus shoot elongation. Dabiski et al. (1979) reported that the addition of NAA to the medium stimulated callus and shoot elongation, while Earle and Langhans (1975) reported that NAA stimulated callus growth but suppressed shoot elongation in carnation.

Impatiens genotypes tested in experiments I and II produced many roots, and NAA had positive effects on root formation between 2-4 uM. Similar results were reported for other herbaceous ornamental plants (Rao et al. 1973, Kunisaki 1975, Schnabelrauch and Sink 1979, and Tamura et al. 1984). Some researchers reported that an interaction between NAA and cytokinin showed better effects on root formation than NAA itself (Rao et al. 1973, Kunisaki 1975, Brandao and Salema 1977, and Dabiski et al. 1979). We observed that 'Starfire' produced abnormal roots which were very short and thick in some treatments of experiment II, while 'T63-1' formed normal roots which were very long and thin. Abnormal roots were at least twice as thick or thicker than normal roots, and root tips of abnormal roots were round and thicker than the basal part of abnormal roots close to the base of the explant. Kunisaki (1975) also observed a similar abnormality of roots during research on in vitro propagation of Cordyline terminalis L. He reported that NAA with higher BA levels promoted the formation of abnormal roots which were very short, thick, and sometimes greatly deformed. However, auxins seem to stimulate root formation in many ornamental plants, and sometimes it is required to induce roots. Otherwise, Stephens (1985) reported that Impatiens wallerana cotyledon explants required the presence of an auxin or cytokinin for root formation, whereas hypocotyl explants of I. balsamina and I. wallerana often rooted without exogenous auxin or cytokinin. He also reported in the same paper that increasing cytokinin levels did not stimulate root formation, and higher cytokinin levels were rather inhibitory. Experiments I and II confirmed these results, showing that

increasing cytokinin levels were inversely proportional to root formation. It was also observed in experiments I and II that there was some relationship between root growth and shoot length. Cultures without roots produced very short shoots and cultures with roots yielded the longer shoots. This seems to indicate that roots are able to synthesize their own hormones in the proper concentration for shoot elongation. Olesen and Fonnesbech (1975) reported the similar results for shoot-tip culture of Phlox spp.

VII. SUMMARY AND CONCLUSIONS

Cytokinins (kinetin : N-(2-furanylmethyl)-1H-purine-6-amine; BA : N-(2-phenylmethyl)-1H-purine-6-amine; 2iP : N^6-(2-isopentenyl-adenine)) and NAA (1-naphthaleneacetic acid) significantly affected *in vitro* propagation of *Impatiens* 'T63-1' and 'Starfire'. BA was most effective for shoot multiplication of 'T63-1', while 2iP was most effective for 'Starfire'. 'T63-1' produced the most shoots at 10 uM BA and 'Starfire' yielded the most multiple shoots at 40-60 uM 2iP. The highest cytokinin concentration, 80 uM, strongly inhibited multiple shoot production of both genotypes. Shoot multiplication of 'T63-1' was not significantly different at any NAA level, while shoot multiplication of 'Starfire' was significantly higher at 2 uM NAA than at other NAA levels at 20 uM cytokinins. High levels of NAA inhibited multiple shoot production, regardless of genotype.

Increasing cytokinin levels inhibited shoot elongation, regardless of genotype. Kinetin at 10 uM was most effective for shoot elongation of 'T63-1', while 2iP inhibited shoot elongation of 'Starfire' with increasing concentrations and kinetin had no effect. All 3 experiments suggest that shoot elongation is related to and inversely proportional to multiple shoot production. As with cytokinin concentration, higher NAA concentration suppressed shoot elongation, regardless of genotype.

BA produced the largest explant diameter for 'T63-1', while 2iP produced the largest explant for 'Starfire'. Explant diameter was not affected much by kinetin for either genotype. Cytokinin concentration

increased the diameter of 'T63-1' as concentration increased, while explant diameter of 'Starfire' was not significantly affected by cytokinin level. Explant diameter of 'T63-1' was not affected by NAA, whereas the explant diameter of 'Starfire' was significantly increased with increasing NAA concentrations. Explant diameter was positively correlated with shoot multiplication and negatively correlated with shoot elongation for both genotypes in all experiments.

Root formation was inversely proportional to cytokinin level. Increasing cytokinin concentration significantly suppressed root formation, regardless of genotype. NAA stimulated root formation significantly for both genotypes tested. However, 6 uM NAA suppressed root formation of both 'T63-1' and 'Starfire'.

Indonesian Impatiens species hybrids can be propagated rapidly in vitro. BA, 2iP and kinetin are 3 cytokinins that are active in causing multiple shoot formation, but at different concentrations. NAA was not effective at the concentrations tested.

VIII. LITERATURE CITED

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